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BREWING

SCIENCE AND PRACTICE

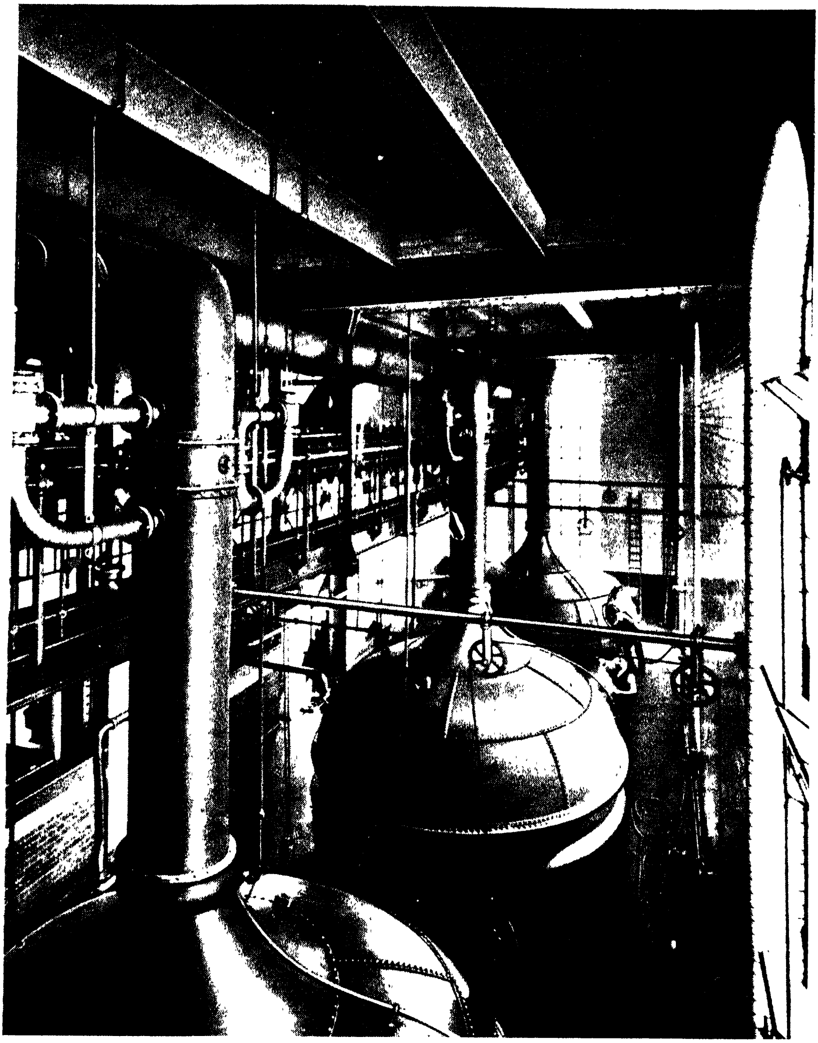

FIG. 64

COPPERS IN A MODERN ENGLISH BREWERY

Frontispiece]

BREWING

SCIENCE AND PRACTICE

By

H. LLOYD HIND
B.Sc., F.R.I.C., F.R.M.S.

VOLUME II

BREWING PROCESSES

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PREFACE TO VOLUME TWO

THE author has been encouraged by the reception given to Volume I of this work to hope that Volume II may meet some of the requirements he set out to fulfil. The results of recent scientific investigations have had such far-reaching effects on current explanations of practical problems and are so suggestive of further possibilities of advance in the elucidation of certain fundamental aspects of brewing that it has been felt desirable to devote space to them which it was originally intended to fill with Chapters on Bottling, Brewery Cleaning, By-Products and Analysis. It is hoped that this will be atoned for by the greater space which it is now possible to allot to Bottling in a third volume. This is now in an advanced state of preparation, with the valued help of a well-known specialist. An attempt has been made to hold the scales even between the description of brewing as an art and the study of operating factors. Some emphasis on the latter should be more helpful in times when changes are inevitable, than any attempt to stereotype well-known methods, however well suited they may have been to the requirements of their time. The writer cannot promise, as did a predecessor at the beginning of last century, that " the Brewer who, by his Attention to the Particulars here recommended, attains the desired Perfection will secure to himself an unsolicited Extension of Trade," but he trusts that readers will agree with the reviewer of 1802 that " he writes like an honest and intelligent brewer and his book will prove useful to those for whom it is calculated," indicating lines of advance to the perfection, regularity and stability of product to which all craftsmen aim.

The friends who so kindly helped the author in preparation of the former volume have continued to give their valued assistance and encouragement, for which grateful thanks are tendered. The help on technical matters given by Messrs. A. J. C. Cosbie, B. Dixon and W. J. Watkins is particularly appreciated. The authoritative Chapter on Bacteria by Dr. J. L. Shimwell, who has made their investigation so peculiarly his own, will be received by readers with as much appreciation as it gives the writer pleasure to acknowledge a valuable contribution to scientific literature. The beautiful series of ultra-violet light photomicrographs of yeasts presented in this volume are magnificent examples of modern technique. It is a melancholy thought that their gift to the author must have been among the last acts of the late Professor Jan Šatava of Prague. The manner in which the Publishers have reproduced them is in keeping with the care they have expended on every detail of production. The result may cover many defects in authorship, but gives the writer a pride in his books. Finally, the author must express his indebtedness to brewing and engineering firms who have so kindly placed photographs of modern plant at his disposal.

H. Lloyd Hind.

325 City Road, Manchester, 15.
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1 Tudor Street, London, E.C.4.

February, 1940

PUBLISHERS' NOTE

It is with the deepest regret that we have to issue posthumously this reprint of Mr. H. Lloyd Hind's classic work. We share with his innumerable friends in the brewing industry a sense of deep personal loss and a full appreciation of all that his untimely death means to the science which he did so much to advance.

The author's corrections to the first edition have been most ably embodied and supplemented by Mr. E. N. Hamnett, whom we thank most cordially for his courteous assistance in this and other directions. Without his valuable help the publication of the revised impression must have been considerably delayed.

Chapman & Hall Ltd.

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CHAPTER XXI

BREWERY EQUIPMENT

EVOLUTION OF METHODS AND EQUIPMENT

(375) 600 Years from 1340.

If the brewer appointed at Queen's College, Oxford, in 1340, were to find himself in a modern brewery of any size, he would be bewildered by its intricate machinery, the multiplicity of thermometers, saccharometers and other instruments undreamt of in his days, and the meticulous care with which every detail of the brews and fermentations was recorded. He would be completely at a loss to understand the reasoning with which his new colleagues explained the nature of their manifold duties, but, having found that an electric motor performed the work he used to do himself, he would soon realise that brewing was fundamentally the same as when he left it. It would still be necessary to mash at daybreak, but something of a shock to find that the wort was in the fermenting vessel by tea time, without the risk of night-long sojourn on the cooler. After that, all would be plain sailing, when he had learnt the respect with which yeast was treated and realised the difference between dealing with hundreds of barrels and five or ten, until he found that beer could be carbonated and filtered to obtain in a week the brilliance he had fearfully awaited through six months or a year. The processes remained the same; the difference consisted in the environment in which they were carried out, the machinery brought into use and the revolutionised outlook on the causes and meaning of the changes from barley to beer. These are illustrated in the frontispieces of this volume and its predecessor, by the photographs of old and new fermenting rooms, and by the three examples of recently erected breweries, Figs. 66, 67 and 68. The spacious steel-framed structures of to-day are a fitting framework for the perpetuation of traditions which have stood the test of time, under conditions of cleanliness and scientific supervision, never dreamt of by the fourteenth century brewer.

(376) The Brewhouse.

Fig. 65 is a generalised sectional elevation, by W. S. Bradford, of a typical English brewery of moderate size, showing, as far as is possible in one plane, the essential departments. The malt room, mill room, grist case, mash room and grains disposal plant are built on a tower system. The liquor tanks command the mash tun from which the wort flows by gravity to the underback and thence to the copper and hop back. Here it is necessary to break the gravity flow and pump hot wort to the cooler or wort receiver, commanding the refrigerator and fermenting rooms, below which are the cleansing rooms, racking room, loading-out stage and

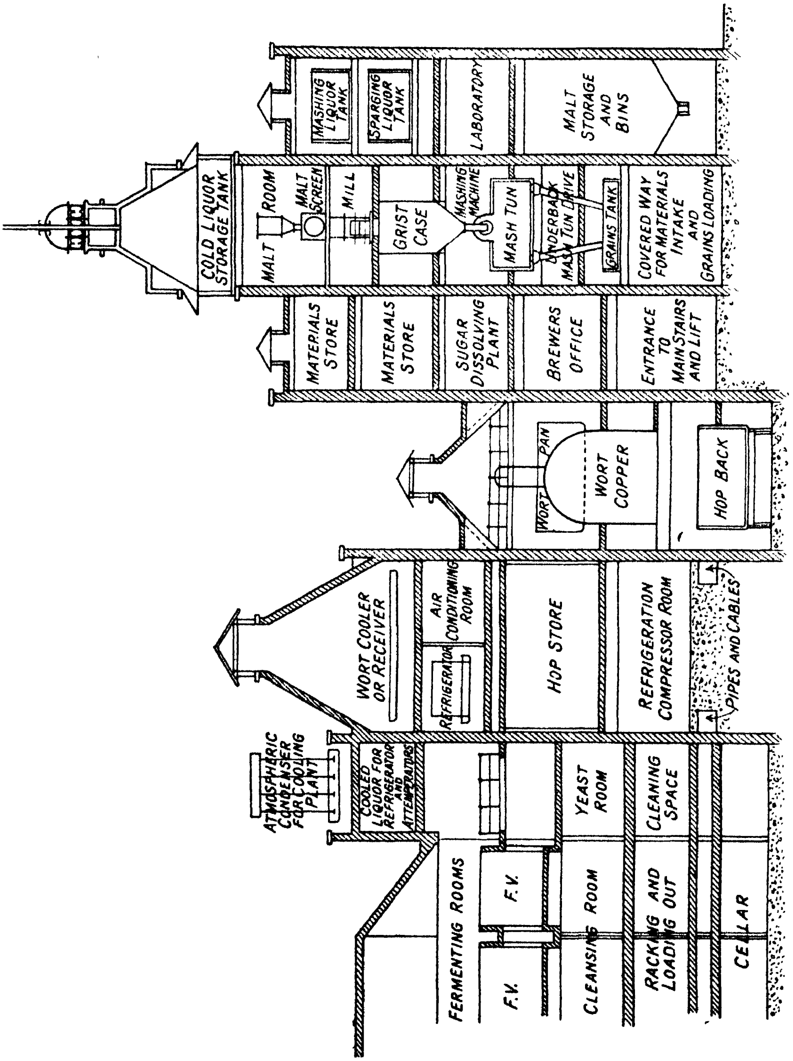

Fig. 65

SECTIONAL ELEVATION OF ENGLISH BREWERY

cellars. The cooling and fermenting rooms are isolated from the brewhouse to avoid, as far as possible, danger of infection from malt dust and spent grains. The steam-raising plant is adjacent to the brewhouse, but no attempt is made to show this or the extensive cask departments. The cask-cleaning plant may be outside the brewery proper, though it is occasionally erected on an upper floor commanding the racking room. Ample accommodation must be allowed for cold and hot liquor tanks, the capacity of which should be sufficient to treat both mashing and sparge liquor. Lager breweries differ mainly in the equipment of the brewhouse, by wide application of refrigeration to fermentation and beer storage, and in the more general use of filters for draught beer.

The building on the right, in Fig. 66, houses malt bins and hop stores. Next comes the brewhouse and, beyond it, the fermenting house. Then follow the administration block and, on the extreme left, the storage building. The by-products plant lies behind the brewhouse, the power house behind the fermenting rooms. The racking, dispatch and cooperage departments are behind the storage building. The storage cellar requirements of bottom fermentation introduce other features in Fig. 67. The brewery consists of a central block, with wings on either side, erected above the fermenting and storage cellars. Incoming casks are dealt with in the building on the left, where washing and pitching machinery is installed. The casks then travel across the bridge to the right wing, which houses the racking plant, with the loading department below. Behind the left wing are the extensive refrigerating and ice-making plants, with the power station. Beyond the picture, to the right, are the maltings, grains drying plant, garage, offices and laboratory, in independent buildings. Fig. 68 is an example of what is being done to reveal the hygienic conditions of a modern brewhouse to the passer-by, a plan adopted, with admirable effect, in a few bottling plants in this country.

Very great improvements have been made during recent years in the construction of malt mills, in the machines for screening and weighing the malt and in the plant for disposal of the dust produced in these operations, as described in the next Chapter. Though there has been little change in the essentials of infusion mashing, very considerable advances have been made in mechanical details of the plant, resulting in more efficient extraction and more accurate control of temperature. The introduction of the mash filter has made it possible to add to the number of brews in 24 hours, though improvements in sparging and running-off arrangements have kept pace in the matter of extracts, at least with well modified malts, while the modern mills deal more adequately with less fully modified material. The evolution of liquor treatment has already been described. It is still largely based on empiricism, but the rough and ready systems, or lack of system, are giving place to accurately controlled softening plants and a more scientific adjustment of hardening treatments, according to the type of beer required.

Development of the triple decoction system of mashing was a masterpiece of empiricism. Ideally adapted to production of the dark beers of Bavaria from coarsely ground, steely malt of low diastatic activity, it owed nothing to knowledge of the influence of temperature on the behaviour of enzymes. The original method of mashing in cold water, followed by a prolonged stand and two thick mash boils, each of an hour and a half duration, were almost essential with malts of that kind and, when later the boils were reduced to half an hour, it became necessary to prolong the boiling of the clear mash to three-quarters of an hour or an hour, to allow time for the crippled diastase to complete conversion of the softened malt remaining in the tun. No thermometers were available when this process was developed, even the iodine test for conversion was unknown. The only guides were the break, the brilliance and odour of the worts, their behaviour on the coolers and in the fermenting vessel and, finally, the clarification of the beer. It was only the closest observation of details and correlation of their effects throughout the entire course of brewing that made elaboration of such intricate processes possible. Science has done no more than endeavour to explain their intricacies and point the way to reduction in the number of mashes and simplification of plant, which are advantageous with pale beers. Introduction of recording instruments and scientific discoveries have proved of immense assistance, but unremitting attention to detail and ability to modify the customary process when unusual results are observed are as essential in brewing as ever they were.

Modern changes in the lager brewhouse have thus been in the direction of simplification, reduction in the number of boils and vessels, and use of the latter for both mashing and boiling. They have, however, been accompanied by development of mechanical and scientific aids to regularity, which might suggest that guidance by external signs was no longer necessary, but this is far from the case. Complete control of mashing will not be possible until means have been developed to measure with greater certainty than is at present possible the inevitable variations in raw material, on which must be based the necessary changes in the mashing process to obtain a product of predetermined

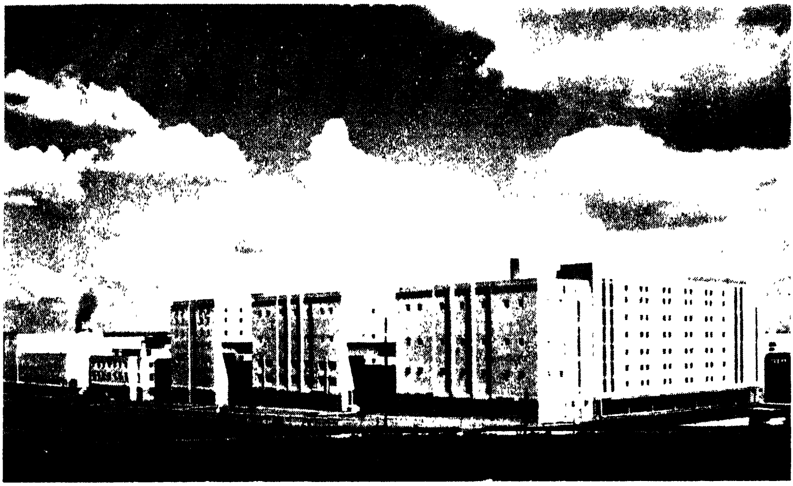

Fig. 66

PARK ROYAL BREWERY (ARTHUR GUINNESS, SON & CO., LTD.)

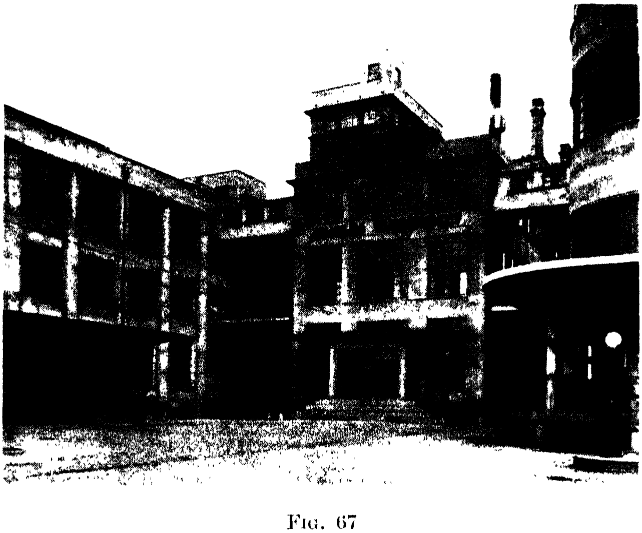

Fig. 67

BRASSERIE CHARLES KLEINKNECHT (SCHILTIGHEIM—STRASBOURG)

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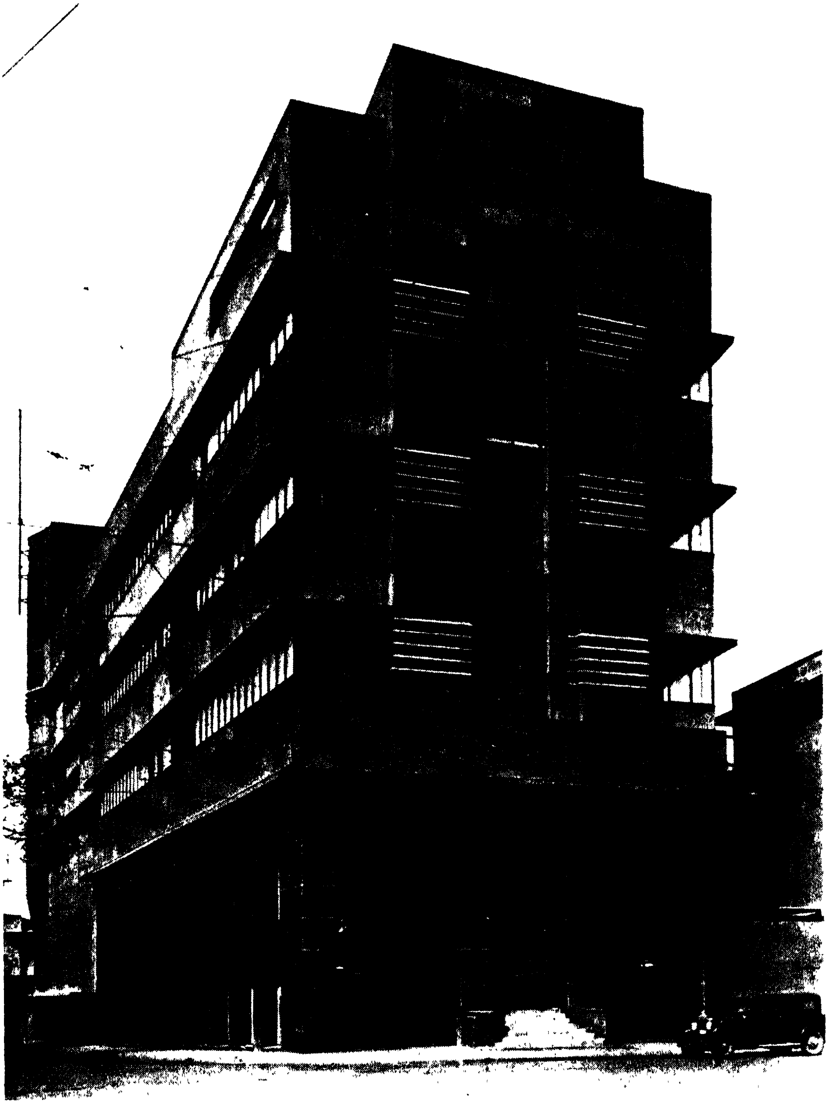

FIG. 68

A MODERN BREWHOUSE (BRASSERIE WIELEMANS-CEUPPENS, BRUSSELS)

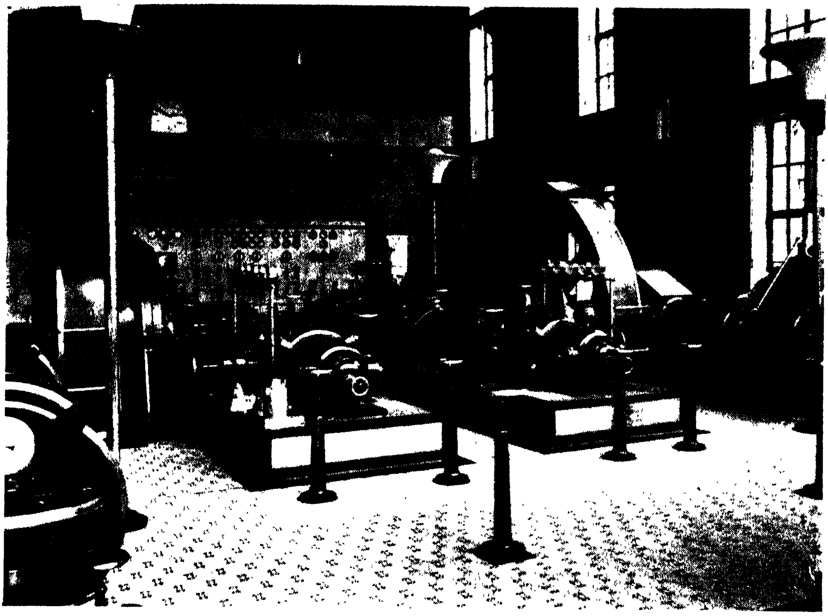

FIG. 69
MACHINERY HALL, LAGER BREWERY (ST. ERIK'S BREWERY, STOCKHOLM)

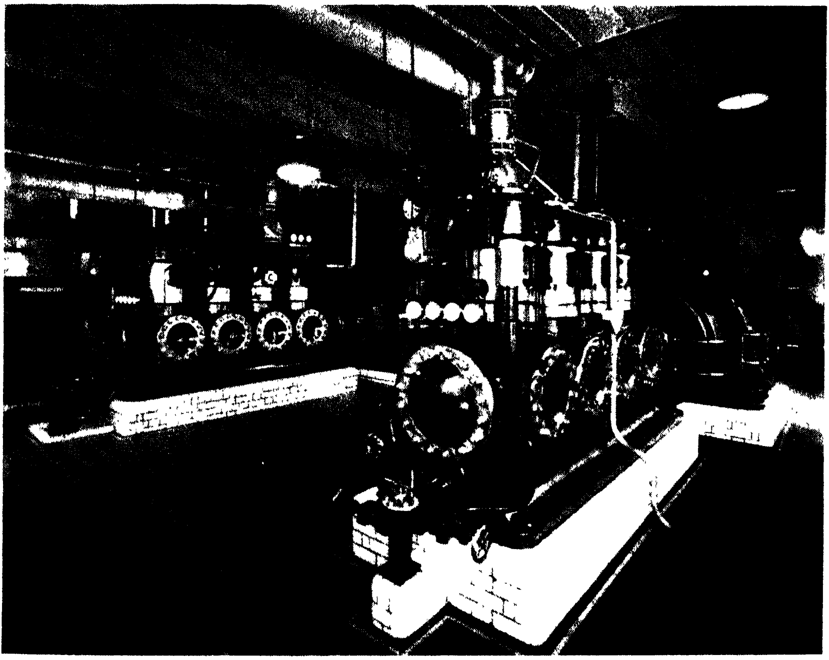

FIG. 70
HIGH-SPEED VERTICAL COMPRESSORS (MANN, CROSSMAN & PAULIN, LTD., LONDON)

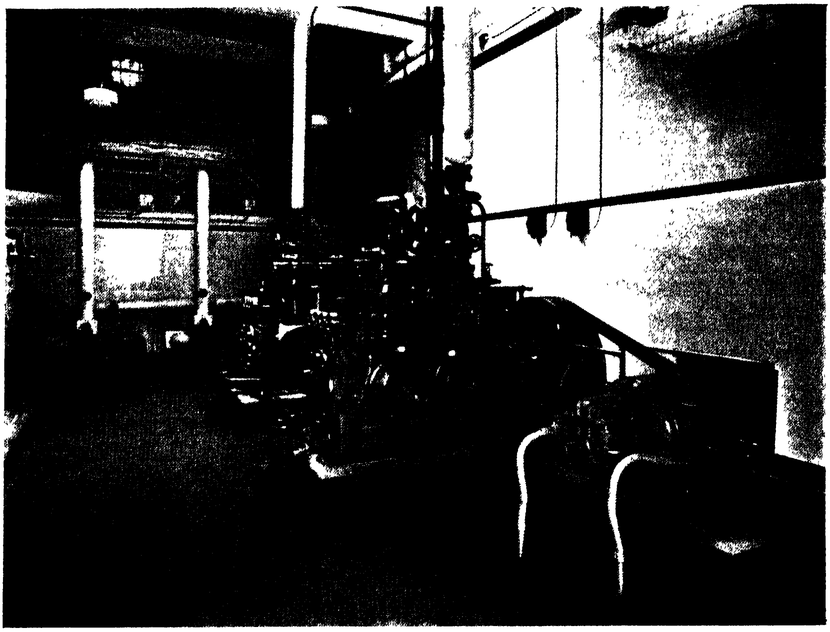

FIG. 71

AUTOMATICALLY CONTROLLED, HIGH-SPEED AMMONIA COMPRESSORS
(BENTLEY'S YORKSHIRE BREWERIES, LTD., WOODLESFORD)

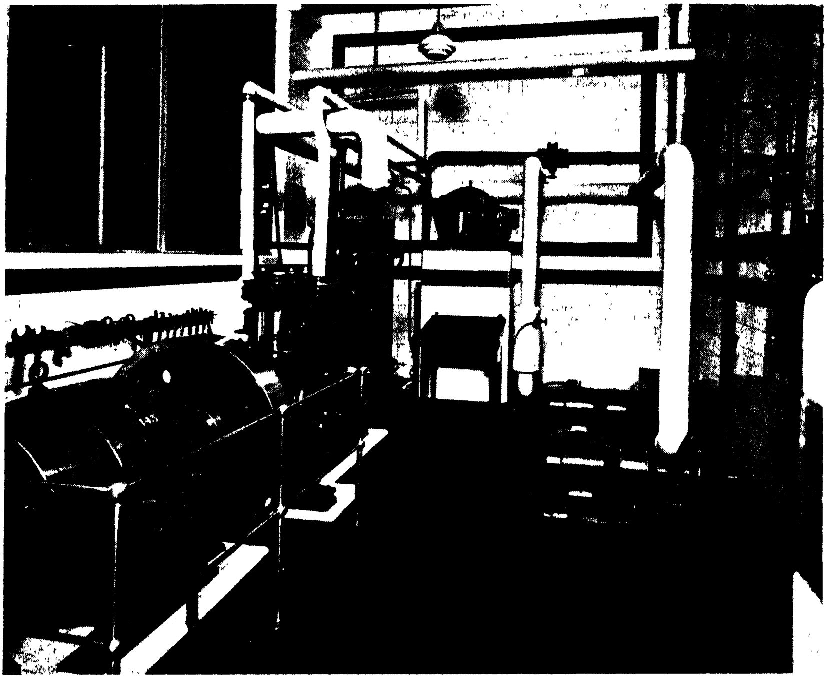

FIG. 72

DIRECT COUPLED, HIGH-SPEED COMPRESSOR FOR HOP STORE
(WILLIAM, YOUNGER AND CO., LTD., EDINBURGH)

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composition. Even then, it would be impossible fully to understand the nature of the changes, unless the problems of the saccharification of starch and of the breakdown of proteins and other constituents of barley were solved. Guidance in mashing is still to a great extent empirical, depending on determination of the amount of apparent maltose produced in the wort, its fermentability and the ultimate stability of the beer. Science has been very helpful in elucidating the effects of temperature, liquor composition and hydrogen ion concentration. Its findings are reflected in methods for adjusting the acidity of the mash and wort. They have made possible striking departures from long-established practice in plant design and methods of operation. Some of these have gone further than the experimental stage and proved successful, either in the direction of economy or of the quality of product, though others have had but a fugitive existence.

Among the former are the Siebel system, designed for use in American breweries in particular, the Nathan and the Wooldridge systems. The essential feature of the first is replacement of the mash tun and copper by two jacketed, insulated, cylindrical steel or glass-lined digesters, fitted with propellers. The malt is finely ground and conversion is carried out in one of these, while the whole mash is filtered and boiled with hops in the other, after which the wort is passed through a section of the mash filter to remove hops, and, at the same time, a second mash can be started in the first digester. Cooling and fermentation are carried out as usual. Details of the other two systems mentioned are given in later Sections.

(377) Boiling and Cooling.

The greatest advance in recent years in the process of wort boiling is to be found in the introduction of steam heating and mechanical devices by which it can be economically adopted. Our knowledge of the changes that occur in the copper is still in its infancy, but great advances have recently been made in investigation of the constituents of hops, as described in Chapters XVI, XVII and XXVIII. The old criteria of a thorough boil and perfect break still remain the guide in practice, but the time of boil has been greatly reduced from the many hours that were formerly thought necessary.

The discoveries of Pasteur and Hansen brought about a revolution in wort cooling methods. They resulted in widespread abandonment of the open cooler and its substitution by enclosed types of wort receivers, and in the development of more efficient refrigerators. Realisation that protection from infection was not the only desideratum and that the cooling process had other functions, brought about renewed demands for open coolers, the satisfaction of which has been made possible by air filtration. The introduction of enclosed refrigerators, in which adequate provision is made for aeration and with which germ-free rooms are unnecessary, has also been most valuable. Sedimentation has been provided for in improved designs or in such sedimentation vessels as that of Nathan.

(378) Fermentation.

Pure Air installations have been more generally adopted in lager fermenting cellars than in top fermentation rooms and, combined with air cooling outside the room or the provision of unit coolers, have led to immense improvement in the design and appearance of the cellars themselves. Realisation of the danger of contact infection resulted in the displacement of wooden fermenting vessels by others constructed of iron, copper, aluminium, stainless steel and concrete. Since attack on metals by beer and the detrimental effect of traces of some metals on yeast had to be guarded against, the introduction of new constructional materials has necessarily been accompanied by the development of impervious and resistant lining materials. Some of these were adaptations and improvements of the brewers' pitch, so long used in wooden storage and trade casks, while others were entirely new enamels or proprietary compositions of the type that has made possible the erection of immense concrete fermenting and storage tanks. Equally valuable was the application of fused glass enamel to steel, which has removed all objections to that metal. These materials have been used with equal success in fermenting and storage cellars and have led to great advances in cellar design, with economy of space through the increased capacity of the tanks. Fermentation in enclosed vessels has been greatly developed, on account of the demand for CO_2 in the production of chilled and filtered beer for draught and bottle.

The expectation felt in many quarters 50 years ago that Hansen's method of pure yeast culture would find general acceptance by ale brewers has not been fulfilled, despite its continually increasing adoption for bottom fermentation beers. At the present time there is, however, a tendency to reopen the question with greater chances of success, on account of the increased proportion of beer delivered from the brewery without any secondary fermentation with what are known as wild yeasts and, in particular, because pure yeast would appear to offer definite advantages with carbonated beer. There have, however, been great improvements in the methods of collection and storage of yeast. Every care is taken to protect it from contamination during skimming, and store it at the most favourable temperature in pure air. In many breweries it is not exposed from the time it is collected from the fermenting vessel by suction until it is again used, after pressing in a refrigerated filter press.

TEMPERATURE CONTROL

(379) Refrigeration.

There are still a number of breweries in Europe where ice is collected during the winter and stored in special ice houses for use during the warmer weather, but the development of lager brewing to its present position would hardly have been possible without refrigerating machinery. This came just when Pasteur's views on the nature of yeast and bacteria were gaining general acceptance. Linde built his first ammonia compression machine in 1873. Since then, knowledge of fermentation and this means for assuring the health and purity of yeast have developed together. Refrigerating machinery is now an essential part of the installation of lager breweries, hop stores and bottling plants and is becoming increasingly used in the fermenting rooms, yeast stores and cellars of top fermentation breweries. The slow running, double acting, horizontal compressor is almost invariably found in the machinery halls of Continental lager breweries, of which an example is given in Fig. 69, but this type is superseded in most modern installations by the more compact, high speed machines, such as is shown in Fig. 70. Steam drive is frequently used for the horizontal machines, and is very economical where a use for the low pressure exhaust steam can be found. The modern type of plant, shown in Fig. 71, comprises two fully automatically controlled, high speed ammonia compressors, driven through tex ropes, and operating on the large brine tank, shown in the background. Cold brine is circulated from this by pumps, each of which is controlled automatically by its individual duty. An example of a high speed, direct-coupled, motor-driven compressor, applied to hop store refrigeration, is given in Fig. 72.

The refrigerating effect of these machines depends on the liquefaction of a gas by compression and cooling, followed by its evaporation under reduced pressure in pipes or other containers where it abstracts the heat necessary for its expansion from surrounding liquids or air. Ammonia is most commonly used for this purpose, but other gases or very volatile liquids, among which are carbon dioxide, methyl chloride and dichloro-difluoromethane or freon, find application under appropriate conditions in breweries. CO_2 requires considerably higher pressures than ammonia. The two other substances mentioned are approximately as efficient as ammonia and are harmless and practically odourless. The continuous cycle of changes from gas to liquid and again to gas could be continued almost indefinitely, were it not for leakage and the presence of water or other impurities. In essentials, the machine consists of a compressor, in which the ammonia or other gas is submitted to a high pressure. The compressed and heated gas is then passed through an oil separator to a condenser, in which it is cooled by water to a sufficiently low temperature to liquefy. The liquefied ammonia then passes through a regulating valve into an evaporator, where it changes back to a gas under reduced pressure, produced at the suction side of the compressor. The condenser, in which heat is taken from the compressed refrigerant, may consist of horizontal pipes and bends over which water is allowed to trickle, or of a shell in which water circulates around ammonia tubes. The evaporator may consist of a coil immersed in the brine, water or beer to be cooled, or of coils erected in the cellar or other room to be refrigerated. The critical temperature of pure ammonia is about 130° C. or 266 °F. and its critical pressure about 112 atm. The liquid boils under atmospheric pressure at —33° to —34° C.

(380) Cellar Cooling.

The majority of fermenting and cold storage cellars are still cooled by coils, through which brine or liquid ammonia is circulated. The latter is known as the direct expansion system and functions by abstraction from the surrounding air of the heat absorbed by evaporation of the ammonia and not by the direct cooling effect of the liquid. Brine pipes are more commonly used than the direct expansion system as they are simpler to control. These systems have a serious defect in that the air of the cellar on cooling becomes nearly saturated with moisture, and water condenses from it on the walls, etc. There is a constant drip of water at temperatures above the freezing point, so that pipes should never be placed above fermenting vessels. The pipes also become coated with frost, which greatly reduces their efficiency and must be frequently removed. Flooded fin type pipes are displacing the ordinary iron coils for ammonia. A back pressure valve operates the coil and prevents frosting. Indirect systems of refrigeration, are, however, preferred for cellar cooling, as they ensure a dry atmosphere without any encumbrance from ammonia or brine pipes. Air is cooled by passage over a battery of brine or direct expansion coils, erected outside the cellar in a special chamber from which ducts can be carried to several rooms, if required. The moisture content of the air is reduced as it is refrigerated and the dew-point is not reached when it is mixed with the warmer air of the cellar. The constant removal of air from cellars by means of fans is also very helpful in keeping the atmosphere sweet and dry.

Many brewers appear to have no objection to accumulations of water on the floors of fermenting rooms, etc., but these may become seats of bacterial infection, while condensed moisture on walls inevitably leads to the growth of moulds and other micro-organisms. Indirect refrigeration makes the problem of cleaning much more simple and should rapidly dry washed floors. The difference between a storage cellar, cooled by air with a relative humidity of 75%, and another with coil refrigeration is remarkable in this respect. In fermenting cellars, there is an unavoidable increase in the CO_2 content of the air, if it is recirculated for the sake of economy in refrigeration. A CO_2 content of 5% is probably the maximum admissible, but it is desirafble not to allow it to rise above 2½%, above which its harmful effects become noticeable. For this reason a proportion of air from outside the building is admitted to the circulation system at the refrigerating coils, to keep the CO_2 content down sufficiently, without unduly raising the temperature. Automatic CO_2 controls have recently been introduced in the circuit, by which a sample of the air is analysed every 10 minutes and the percentage of CO_2 registered. These admit the necessary quantity of air to maintain the CO_2 content between 1½ and 2½%.

The problems of insulation cannot be discussed here, but no method of cooling can be economically adopted unless transmission of heat through the walls, floors and ceilings is satisfactorily prevented.

(381) Air Conditioning.

The application in breweries of modern methods of air conditioning has made it possible, in large measure, to overcome difficulties formerly inseparable from warm weather, and to exclude air-borne infection, while they have introduced improvements in working conditions that cannot fail to be of advantage to the health of employees, and in the general cleanliness of plant. These advantages are particularly marked in the stabilisation of temperatures and hygroscopic conditions in fermenting rooms, yeast rooms, hop stores, storage cellars, cooling and refrigerator rooms and cask beer stores. Purification and control over the moisture content of the air is of the greatest help in keeping the brewery clean, and the removal of CO_2 greatly increases the comfort of working in fermenting rooms and cellars. The introduction of unit systems has made it possible to satisfy the very varied requirements of the different departments in a way that was quite impossible with the older refrigerating systems. Air conditioning involves:

(1) Control of temperature.
(2) Maintenance of the proper relative humidity.
(3) Distribution and control of air movements.
(4) Air purification.

How these should be applied, which are requisite in each particular department, and the conditions which it is essential to maintain are the concern of the brewer. How his requirements are to be met must be decided by the engineer. The conditions in a brewery are so different from those which apply to buildings where comfort and health are the only considerations, that co-operation between brewer and engineer is essential if full advantage if to be taken of air conditioning. There must be ample refrigerating capacity to deal with the maximum external temperature, which usually coincides with the greatest output of internally generated heat in fermenting rooms, where each pound of maltose fermented gives off about 280 B.Th.U. In some cases it is necessary to dry the air, in others, to humidify it.

Air filtration need not be considered in every case, but is usually advisable in cooler, wort refrigerator and yeast rooms and often in the fermenting rooms. The problem of doors and other possible sources of air inflow is not so difficult as might be imagined. An essential point with all installations, whatever their application, is that a slight over-pressure or plenum should be maintained in the air-conditioned space. This pressure is so slight that it cannot be measured by an ordinary gauge, but it must be sufficient to cause a definite outflow of air. This makes it unnecessary hermetically to close the rooms. Ordinary doors and partitions are quite adequate and there is little danger of back-flow through unavoidable openings. It is usual in cold rooms to conduct the air through suitably disposed outlets to the refrigerating unit for re-circulation.

Temperature conditions for top and bottom fermentations are very different, but stabilisation is almost as helpful in the former as in the latter. A constant temperature of about 40°–43° F. is commonly adopted in lager breweries, while the most favourable temperature in top fermentation rooms may vary between 48° and 62° F. Though 48° proves quite suitable in some breweries, it may detrimentally affect the fermentations in others, the difference depending on the materials of the fermenting vessels and the activity of the yeast. 48°–50° F. may be permissible with large wooden vats, while 55° may be the lowest applicable in rooms with metal vessels. An important advantage of air conditioning in top fermentation rooms is the help it gives in keeping down the temperature of yeast heads. This is always higher than that of the wort and is a factor in yeast deterioration. Provision must be made for heating in the winter in many top fermentation rooms and automatic temperature controls are available for this.

With adequate thermostatic control, it is possible to maintain the temperature of storage cellars within 1° or 2°, which is very desirable where beer is matured at 32° or 33° F. A point that may be overlooked in the refrigeration of storage cellars, is that the air enters the tanks when they are emptied and in consequence air filtration is advisable. Air currents on to tanks must be avoided, but the advantage of adequate circulation is very marked in the rapid drying of the floors after tanks are washed. This advantage cannot, however, be secured if the vessels are of wood which is liable to damage through desiccation. The air in yeast rooms should always be kept at a constant temperature and be purified by filtration. In lager breweries the temperature is kept near to the freezing point of water, but the optimum temperature for storage of top fermentation yeasts varies and may be as high as 45° F. for unpressed yeast. Pressed yeast is usually stored at about 32° F.

The conditions in cooler and refrigerator rooms are different from those in the fermenting rooms. Prevention of condensation and drip are just as essential there as pure air. It is, for this reason, necessary slightly to warm the air and reduce its relative humidity, but it is not always a simple matter to get the best balance between these two factors. A temperature of about 90° F. is generally suitable for cooler and refrigerator rooms. Washing and cooling elements are not essential. A filter and heater are all that is necessary. Cold air is required in the hop store to minimise the changes due to micro-organisms and enzymes. A certain amount of dehumidification is desirable, but this must not be so great as to dry out the hops. Purification is probably not necessary. Maintenance of an equable temperature in cellars where cask beers are kept previous to dispatch is one of the most valuable applications of air conditioning. It prevents a large proportion of the frets and summer troubles that afflict top fermentation beers. The requirements are similar to those of the hop store, except that the temperature is kept at about 55° F.

Applications of air conditioning are described in the Sections on cooling and fermentation. The requirements under various conditions are summarised in Table 151, air filtration being added wherever it may appear desirable.

TABLE 151.—AIR CONDITIONING REQUIREMENTS

	Temperature, Fahrenheit	Relative humidity, %
Cooler and refrigerator	90°–115°	30–40
Fermenting rooms:		
Top fermentation	55°–62°	75
Bottom fermentation	40°–43°	75
Yeast stores:		
Top fermentation, liquid	40°–45°	—
„ „ pressed	32°	75
Bottom fermentation	32°	—
Cold storage cellars	30°–33°	50–70
Ale stores	55°	75
Hop stores	33°	75

(382) Conditioning Units.

There are several types of conditioning units, applicable to varying conditions and differing in the refrigerating and air purifying arrangements. A suitable plant for top fermentation breweries consists of air filters, air washer and cooler, air heater and fan. In one type, the filtered air is drawn upwards through a bed of wood wool or fine shavings and sufficiently cooled by a fine spray of cold liquor, which percolates through the bed to a copper tray below. The cooled air then passes through a second bed of wood wool above the sprays, where the superfluous moisture is removed, though the air remains in a saturated condition. It is then generally desirable partially to dehumidify the air, in order that it may act as a drying agent in the room. For this purpose, it is further cooled by a small refrigerating coil in the casing. The moisture-absorbing power of the air is thus reduced, with the result that a proportion of that held in it at saturation at the higher temperature is condensed out of it. The cooled and dehumidified air is then heated again to the required degree by passing over thermostatically controlled steam heaters in other casings. In this way it is possible to deliver air at a constant temperature and predetermined moisture content or humidity. The rate of air delivery, or capacity of the unit and fans, is based on the space to be conditioned and the number of times the air is to be renewed per hour, as well as on the peak load to be dealt with. It may, for example, be considered desirable to renew the air of a fermenting room 9 times per hour during the day and 6 times per hour at night. In the air purifying unit, shown in Fig. 73, this is effected by two motors of different horse power, which may be coupled at will to the same fan, so that it shall deliver either 9,500 or 6,000 cubic feet per minute.

A completely automatic conditioning unit of another type, installed in a fermenting room, is illustrated in Fig. 74. Air is drawn through this installation by a fan at the delivery end, to the left of the photograph. The room itself serves as the return duct, air entering through the grid on the right to a chamber, where it is mixed with an adjusted proportion of fresh air, drawn in through a duct. The air then passes successively through chilled water sprays, moisture eliminator, two sets of viscous type filters (Section 385) and heater to the fan and delivery ducts. A sufficiently low CO_2 content is assured by adjusting a damper in the fresh air duct. If, for example, the air of the room is changed ten times per hour, the fresh air may be admitted at the rate of one-tenth this volume. The water sprays in the next compartment are supplied by a pump from a tank below, in which the water is chilled to 36°–38° F. by brine coils, served by a refrigerator. This water is returned from the chilling chamber to the tank, its refrigeration being automatically controlled by a thermostat in the filter chamber. Excess moisture is then removed from the air by the eliminator, before it passes through the two sets of viscous filters. Each of these consists of 24 plates. Two of those in the first filter are replaced every three days, so that the whole filter is renewed once every 36 days. The plates in the second filter require replacement much less frequently. The chilled and filtered air must then be warmed to the temperature required by passage through a steam or electric heater, which is automatically controlled by a thermostat in the fermenting room.

Ducts should be reduced to a minimum in air-conditioned rooms and, wherever possible, the air should be delivered through openings in the walls or ceiling. This course has been adopted in the modern bottom fermentation cellar shown in Fig. 75. The rooms should always be finished in such a way that the floor, walls and ceiling are waterproof, so that they can readily be washed and sterilised, while all avoidable encumbrances are eliminated. The floor, walls and ceiling of the yeast room, illustrated in Fig. 76, are finished in asphalt, the walls and ceiling being white-enamelled. This method of treatment has many applications, and is very durable. The room shown in the photograph is long and narrow, with two doorways. Temperature is thermostatically controlled and the moisture content of the air reduced by passage through a calcium chloride spray. This entirely prevents the growth of mould. The air delivery duct is on the left, the outlet on the right. The doors are, in addition, fitted with vanes which open outwards under the slight pressure established by the air circulation. Their function is to remove the CO_2 which accumulates near the floor of a yeast room.

Though the temperature in bottom fermenting cellars may have to be kept at about 40° F., the problem of adapting unit coolers is generally not so difficult as in top fermentation rooms, where less attention had been paid to insulation or regularity of temperature when they were built. Suitable units consist of one or more coils, together with a fan of adequate capacity, air filters and automatic controls in a sheet metal housing at one end of the room, near the ceiling. The coils are fed with refrigerated brine, with regulating valve for automatic control of the room temperature, the fresh air inlet being fitted with a hand controlled damper. A small heater is placed in the fresh air inlet, for use in the winter if the outside temperature falls below 40°.

(383) Delivery of Conditioned Air.

Correct distribution of conditioned air is particularly important in fermenting rooms, where currents of cold air, directed on to fermenting wort or the sides of fermenting vessels, must be avoided. Wooden vessels may be seriously damaged by a current of relatively dry air and the effect of chill through metal vessels may be very detrimental. The conditioned air is distributed, at a velocity not greater than 75 feet per minute, through conduits in the wall itself or through insulation board ducts of rectangular section, along the ceiling, at either side of the room. This material does not corrode and is more durable than galvanised iron. The ducts are fitted at intervals with slotted openings, fitted with adjustable deflectors to distribute the air in such a way that it forms a blanket extending across the ceiling to every corner of the room and, descending with reduced velocity, displaces the CO_2-laden air of the room downward to louvres near the floor, whence it is carried through ducts to the conditioning unit, where it is again filtered, cooled and dehumidified. It is then recirculated, after mixing with an appropriate quantity of freshly conditioned air. The quantity of air passed over the refrigerating coils must be carefully adjusted. Too much results in high relative humidity, with draughts and lack of drying ability. Too little means excessively low temperature and low relative humidity.

PURE AIR

(384) Aerial Infection.

The natural winter habitat of yeasts is the soil. In damp

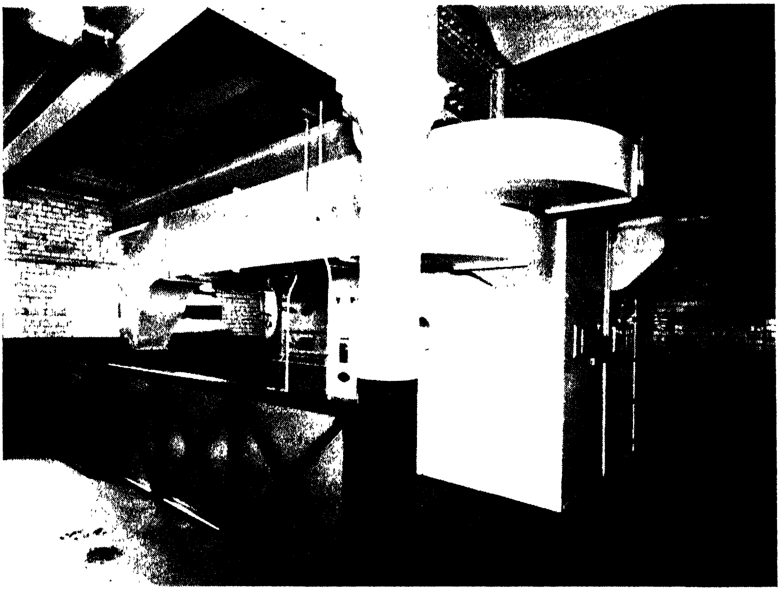

Fig. 73
AIR CONDITIONING UNIT FOR FERMENTING ROOMS
(MAGEE, MARSHALL AND CO., LTD., BOLTON)

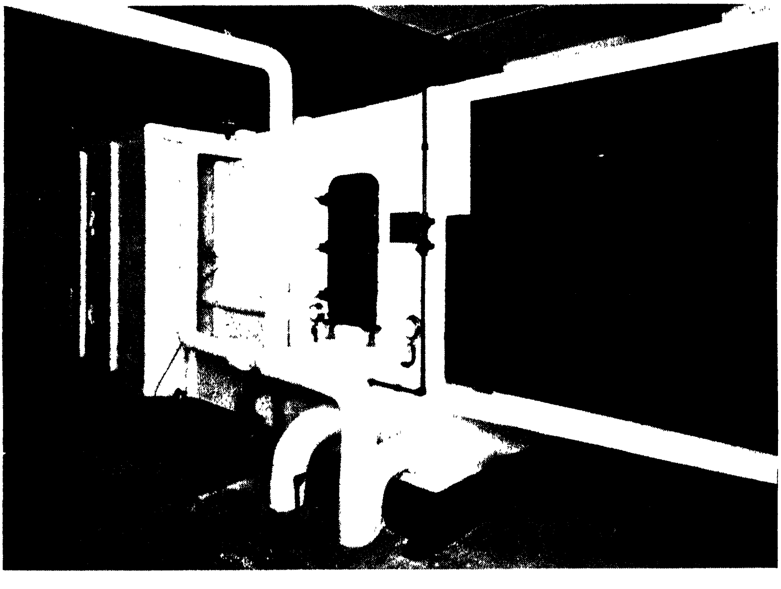

Fig. 74
AIR CONDITIONING UNIT FOR FERMENTING ROOM
(BENSKIN'S WATFORD BREWERY, LTD., WATFORD)

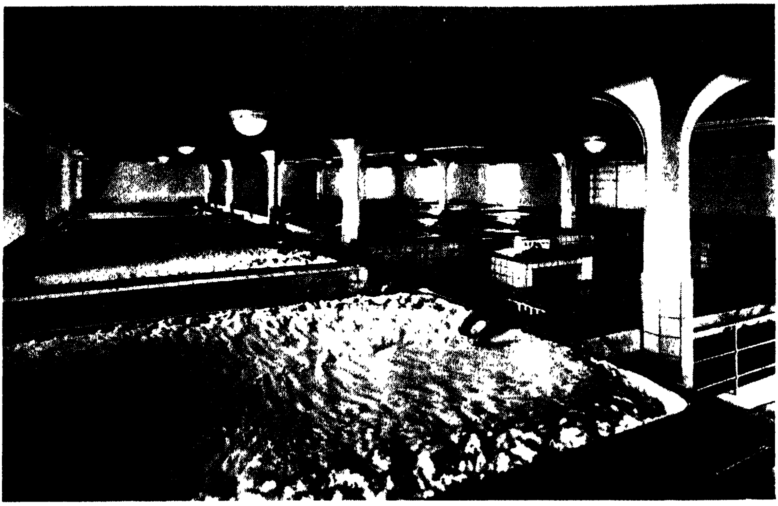

FIG. 75
AIR-CONDITIONED FERMENTING CELLAR
(LUZERNER BRAUEREI ZUM EICHHOF A.G., LUCERNE)

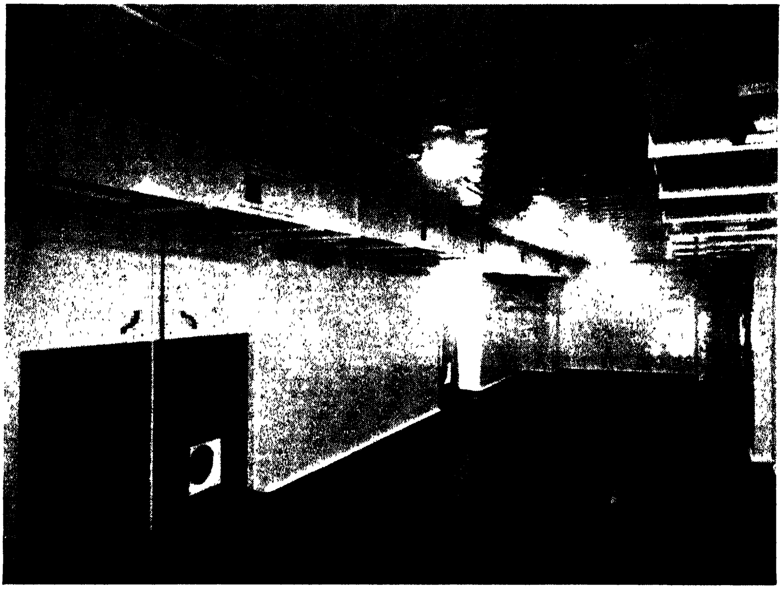

FIG. 76
AIR-CONDITIONED YEAST ROOM (J. W. GREEN LTD., LUTON)

meadows the cells may persist in a vegetative state, but their activity is greatly reduced in dry places and spores may form. Dust, with attached cells and spores, may be carried by the wind and deposited anywhere. Insects carry them to ripe fruit and deposit them in their excrement. Wort is an equal attraction to insects and hence may arise sporadic developments of wild yeast in beer, particularly at times dependent on the ripening of fruit. Similarly, flies may carry infection from other external sources of yeast nutriment, such as refuse heaps. Covering windows and vessels with muslin has been successfully adopted as a protection in some instances, while relief, in others, has been secured by removal of the external source of contamination. Infection of this kind is far from confined to yeasts. Bacteria and moulds may equally well be present and, though insects may in some cases provide the major means of transport, dust may be the main cause of infection in others.

Morris[1] made the rather alarming calculation from experiments at two breweries that 140,000 organisms fell on each square yard of cooler surface per hour, which is equivalent to about 250 on a Petri dish of 10 square inches area, in 15 minutes, and the even more alarming calculation that cooled wort taken from the coolers when all but a few barrels had run down contained, in the one case, 163,548,000 and, in the other, 1,406,512,800 germs per barrel capable of growing on wort gelatin. These figures represent about 1,000 and 8,600 germs per ml. The nature of the organisms found in air varies according to the weather, their numbers being greatly diminished by rain. Bacteria are most abundant in dry weather. Moulds occur in greatest quantity in damp weather and yeasts from June to August or during the time of fruit ripening. Their number in the air is commonly ascertained by counting the colonies that develop on wort gelatin or wort agar in Petri dishes exposed for 10 or 15 minutes. Nutrient agar with a p_H value of 6·6 is a better medium for some organisms that will not develop on the wort gelatin or agar at lower p_H values. Petri dish exposures should be regarded merely as a means for ascertaining the degree of general infection, because so many bacteria are unable to develop or are swamped by moulds and yeasts. A heavy growth of micro-organisms does not necessarily mean that any of them can cause trouble in beer, but should be regarded as a warning of potential danger. More reliable counts can be obtained by slowly aspirating 5 litres of air through a small quantity of liquid, of which an aliquot part is plated in a suitable nutrient medium.

Air filtration has become very general as the most convenient protection against aerial infection, but experience has shown that the danger is slight in more favoured localities, where sufficient protection on the cooler is assured by the high temperature of the wort. It is now realised that the greatest danger in all cases arises from contact infection through neglected plant, accumulation of wort and beer residues or drip from steam condensations on ceilings and beams. An instructive example of the effect of this change of view on modern cooler construction is presented in Fig. 77, from a brewery not far from that in which Hansen carried out his classical work. These coolers are constructed of stainless steel, a material that can be kept scrupulously clean, housed in a room where the danger of condensation and drip is eliminated and open to currents of air through windows that can also be kept clean. According to Claussen's[2] calculations, on the basis of 7 germs falling on a Petri dish 63 sq. cm. in area as representative of the purer air in more favoured localities, 1 ml. of wort cooled for 5 hours would contain 0·2 germs. This is quite an insignificant number, compared with the infection from a yeast containing 2 bacteria for every 100 yeast cells.

Adrian Brown calculated that a pound of English barm contained 724,800,000,000 yeast cells. If this was added to a barrel of wort and carried 2% bacteria, the wort would contain 14,496,000,000 bacteria per barrel or 88,576 per ml. Claussen further stated that, in his experience, 5,000 to 10,000 germs per ml. was a fair average for wort entering the fermenting vessel. In most cases air-borne contamination is less significant than the figures for colonies on Petri dishes represent, since the majority may be harmless mould spores and, of the rest, most would be so enfeebled by desiccation as to have no chance of survival in a fermentation that started rapidly. The decision for or against air filtration in particular circumstances should be guided by the number and nature of the organisms found by air analysis throughout the year and the vulnerability of the wort, yeast or beer to be protected. The cooler is not the greatest danger spot in the brewery. Others that are liable to be overlooked, such as primings making plant and filter pulp, require even greater protection.

(385) Air Filtration.

If air filtration is held to be desirable, it becomes necessary to consider how it may be most satisfactorily effected under the existing circumstances. Passage of air through a spray of calcium chloride brine in wet systems of refrigeration has a sufficiently purifying effect to satisfy many requirements, although the delivered air is not sterile. The most probable defects in such installations are rust in the air washer and failure completely to eliminate the spray, which is then carried into the ducts in the form of a mist. In dry refrigerating systems, the air is cooled by passage over finned brine coils. These are satisfactory if the cooling surface is adequate to prevent the formation of frost from air of relatively high humidity but do not purify the air. In any case, it is necessary to instal air filters if sterilisation is required.

Both dry and wet filters are used. Cotton wool, packed in cylinders, finds its applications, and a reasonably efficient filter can be made by packing a wooden frame, two or three inches in depth, with kapok. Antiseptic solutions, such as permanganate, were formerly frequently used, in conjunction with cotton wool filters, for washing air. Cabinets, fitted with trays coated with glycerin on which micro-organisms settled and were trapped, as the air circulated between them, were also used, but these simple methods of filtration have now given way to specially woven filtering fabrics, to viscous filters or beds of porous material wetted with glycerin. The viscous type filters consist of frames, packed with metal ferrules or some type of corrugated baffle plates, covered with oil to retain the micro-organisms, and depend for their efficiency on splitting the air into the smallest particles possible and the eddying effect produced as it is drawn through the baffles. In any case the filters sooner or later become dirty and fail to sterilise the air, so that means must be provided for renewing them from time to time. Air purity tests should, consequently, be made frequently by exposing Petri dishes or by other means.

The plant shown in Fig. 73 comprises a double set of fabric pockets in casings of non-corroding and non-condensing material. Each pocket is independent and is kept at the correct tension by spring bolts, which take up the expansion or contraction of the cloth caused by changes in temperature or humidity. They have a very large filtering surface and only allow the air to pass at a low velocity. It is usually necessary to remove one set for cleaning at intervals of 4 to 6 months. Cloths can thus be replaced without discontinuing the supply of pure air. Filters of the viscous type, similar in principle to those fitted in the unit illustrated in Fig. 74, are shown in Fig. 80, as applied to air purification in a refrigerator room (H. R. Henius[3]). In this case they are used for final sterilisation of the air, after a preliminary filtration through cotton wool.

The air conditioning unit shown in Fig. 78 has a capacity of 8,000 cubic feet per minute, though the requirements are much less. The air drawn through a large screened duct, connected with the window, is passed through a bank of cotton filters, Fig. 79, to remove dust. These are repacked after about 72 hours use or 24 brews. Beneath the filter is an air-heating unit, consisting of herring-bone steam coils, with thermostatic control for keeping the air at 90° Fahr., which is required to prevent steam condensation in the room. Manual control of these has, however, proved most satisfactory.

The air is finally purified by passage through the oil-coated filter, shown in Fig. 80. This consists of 4 frames, 20 inches square by 4 inches deep. These are constructed of double crimped, galvanised iron, woven into a suitable pad and coated with refined, tasteless and odourless, Russian mineral oil, on which the micro-organisms adhere. Filtration is most efficient when operating at maximum capacity of 800 cubic feet of air per minute through each frame. The filters can be used for about 30 brews or 90 hours, after which they are cleaned by high pressure steam, dried in air and immersed in the oil for 20 minutes. They are then allowed to drain for 24 hours, wiped of surplus oil and replaced.

The air purifier shown diagrammatically in Fig. 81 consists of a galvanised, mild steel container, made in sections, each of which is fitted with a perforated tray, carrying a bed of filtering material, packed to a depth of 12 inches and soaked with glycerin. This arrangement ensures that the air is very finely divided and brought into intimate contact with a large viscous surface. Glycerin is stored in the reservoir at the base of the machine, and is pumped through a series of sparge pipes, to saturate each bed. The glycerin drains very slowly into the reservoir and, when a marked point on the sight glass is reached, is again pumped over the beds. Potassium metabisulphite is introduced periodically into the glycerin, in order to maintain it in an aseptic condition. A container for this purpose is shown in the glycerin supply pipe. The glycerin must be maintained at a specific gravity of 1·22–1·24, for most efficient results. A considerable drop in specific gravity occurs during normal operation, on account of its hygroscopic property. Air passed through glycerin, at a temperature higher than that of the latter, will, however, absorb any moisture that it may have taken up. The fall of gravity can, consequently, be compensated by installing an air heater between the fan and filter. This may be used continuously when warmed air is required in a refrigerator or cooler room, but the moist, warmed air must be passed out of the circuit through a by-pass, if cold air is needed. Air conditioning would then be temporarily stopped, while the glycerin was being revivified.

When the air to be purified is more than usually dirty or infected, it is advisable to pass it through a water spray washer before delivery to the filter. Refrigerating plant is placed in a similar position when cold air is required. Brine sprays are used, if temperatures below 32° are necessary, and dry refrigerating coils for higher temperatures.

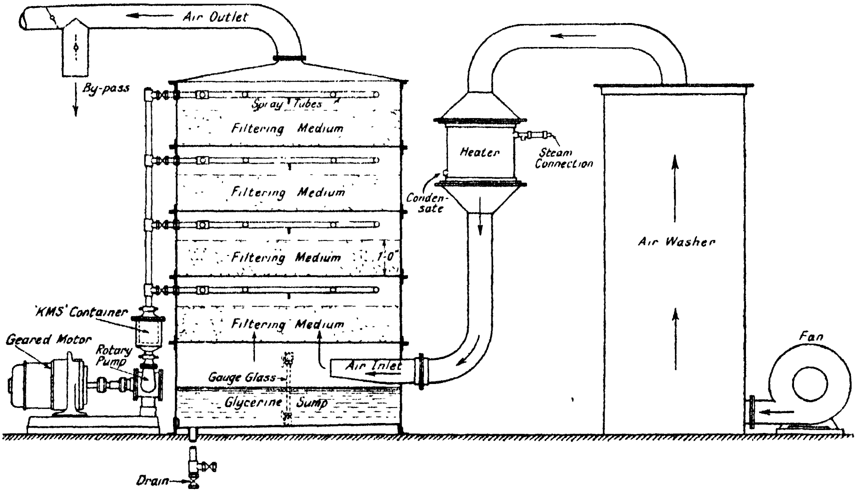

FIG. 81

AIR PURIFIER WITH GLYCERIN FILTER BEDS

(386) Summary.

The introduction of refrigerating machinery in the last quarter of the nineteenth century marked the beginning of a new era in brewing. Coming at the same time as Pasteur's discoveries, it provided the means whereby the health of yeast and the regularity of fermentation could be assured. Realisation of the danger of aerial infection followed almost immediately, and air filtration was soon taken up as the obvious means for avoiding the difficulties which had resulted, in some breweries, from abandonment of open coolers. This naturally led to the combination of refrigeration and air purification, but many years were to pass before this attained its present stage of development in air conditioning units. Pure air is generally synonymous in breweries with germ-free air, though water vapour, extraneous vapours and non-living solid particles should also be regarded as impurities. They do not impair the stability of beer, but offensive odours may be absorbed, and a high relative humidity preserves the vitality of micro-organisms which might become innocuous in dry air. It is in damp, hot weather that air-borne yeasts and bacteria are most to be feared. Air purifying plant is capable of dealing with all kinds of impurities. Offensive vapours can be removed by washing. Water vapour, whether originally in the air or added by washing, can be "squeezed" out of the air by refrigeration, so that its relative humidity is greatly reduced on re-warming.

The danger of aerial infection is, in general, much less than that due to micro-organisms established in the plant. Air filtration can afford comparatively little protection when such sources of contamination are allowed to remain. It must be accompanied by such structural arrangements as will permit of scrupulous cleanliness in the rooms and plant, will preclude the ingress of dust from all external sources of infection, and remove the possibility of drip from ceilings or beams where micro-organisms may lodge, despite all care. Purified air is then driven in to maintain an imperceptible but definite pressure in the room, so that little danger arises when doors are opened for essential purposes. Air conditioning, particularly by reducing the relative humidity of the atmosphere, is then of the greatest assistance in maintaining cleanliness, provides a strong urge to sterilisation of utensils, in which germs are so easily carried, and, in addition, is very beneficial in preserving the buildings and plant. Much can be done to improve the conditions in old rooms, encumbered by beams and pipes, designed before much attention was given to brewery hygiene. Vessels may be covered and supplied with pure air. Smoothly surfaced ceilings may be installed, and the pure air ducts carried above them, with suitable inlet and outlet apertures. Windows, doorways and stairs can be suitably screened.

Introduction of new constructional materials and improvements in the design of rooms and cellars have kept pace with the development of pure air systems, so that wort cooling, fermenting and yeast rooms have been completely transformed. Condensation and drip are abolished, with the elimination of unsightly accumulations of water and wort, which had formerly served as traps for rapidly growing micro-organisms. Refrigeration also made possible the rapid expansion of bottled beer trade, and there are signs that its more general application to the sale of draught top fermentation beer may be equally successful.

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2. N. H. Claussen, *Master Brewers Association of America*, Convention, 1936.
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WORT PRODUCTION
AND
COMPOSITION

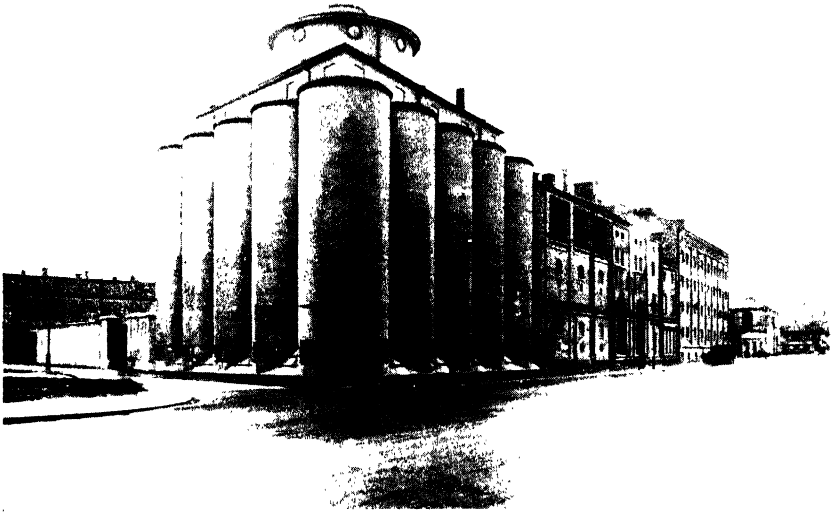


Fig. 82
MALT SILOS (PRIPP AND LYCKHOLM, GOTHENBURG)

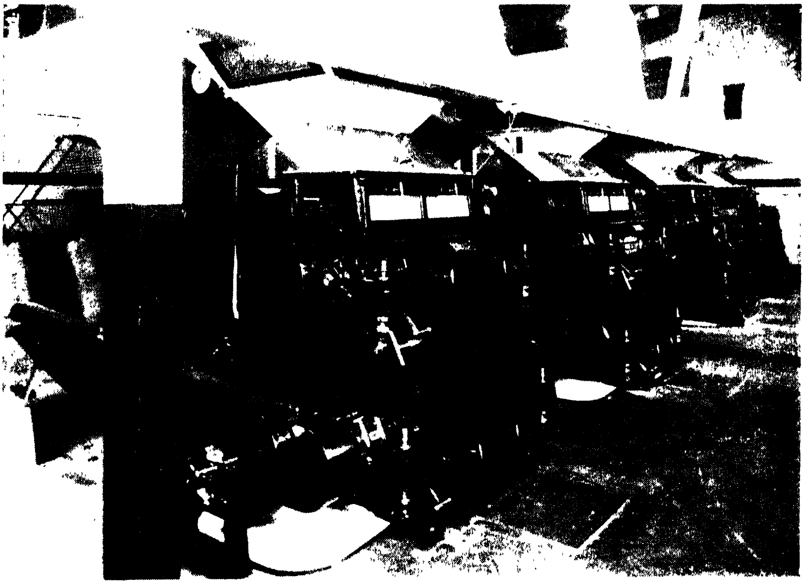


Fig. 83
MILL ROOM (WHITBREAD AND CO., LTD., LONDON)

CHAPTER XXII

THE GRIST

(387) Malt Storage.

Many brewers find it convenient to store malt in steel or reinforced concrete silos of several hundred quarters capacity. This almost inevitably leads to bulking malts of varying composition or character, a very serious disadvantage, in view of the difficulty of maintaining regularity of wort composition under such circumstances. Silos of this kind, generally of steel, may be built inside the brewery or constitute a separate building, when they are usually made of concrete. A building of this kind is shown in Fig. 82, the internal construction of another being illustrated in Fig. 84. The concrete, in this case, is smoothed on the internal surface, but not lined with plaster or any other material likely to flake off into the malt. The silos are protected externally with pitch or Mammut and malt takes up very little moisture during a season's storage. Malt is usually fed into the silos by a pneumatic system, through a distributor above them, and may be delivered from the conical bottom of the silos to the mills in a similar manner or by means of mechanical conveyors.

The malt room of English breweries is generally above the mash room and conveniently situated in respect of the mills. Malt is usually received in small quantities in sacks, so that the identity of different strippings or bins can be preserved. The temperature of these rooms is commonly kept at between 80° and 85° Fahr. The relative humidity of the air should be low and it is very necessary to take precautions to prevent the ingress of steam or damp air, otherwise the cooler malt in the sacks will absorb moisture very rapidly. It is preferable to store the malt in steel or concrete silos, of sufficient size for a few days' consumption only.

The risk run by storing malt for a short time in a warm room is not great, in so far as deleterious changes resulting from the condensation of 1 or 2% of moisture is concerned, but an increase of moisture content leads to an apparent loss of extract, if the malt is weighed for the mash after condensation has taken place. Warm air will carry a considerably greater quantity of moisture, without condensation, than cold air, as shown in Table 152, and that of a malt room may well give a sensation of dryness, despite its greater moisture content, than the cooler air of an adjacent room. The hygrometric state of air is expressed in terms of "relative humidity," which is its moisture content as a percentage of the quantity of water it could hold when saturated at the given temperature. This percentage is reduced when cold air is warmed without any addition of moisture, but it frequently happens in malt rooms that there is some unobserved access of steam causing an increase of humidity. The temperature of the air in contact with the colder sacks of malt is reduced, until it may become saturated and condensation commences. This is rapidly absorbed by the malt, which is very hygroscopic.

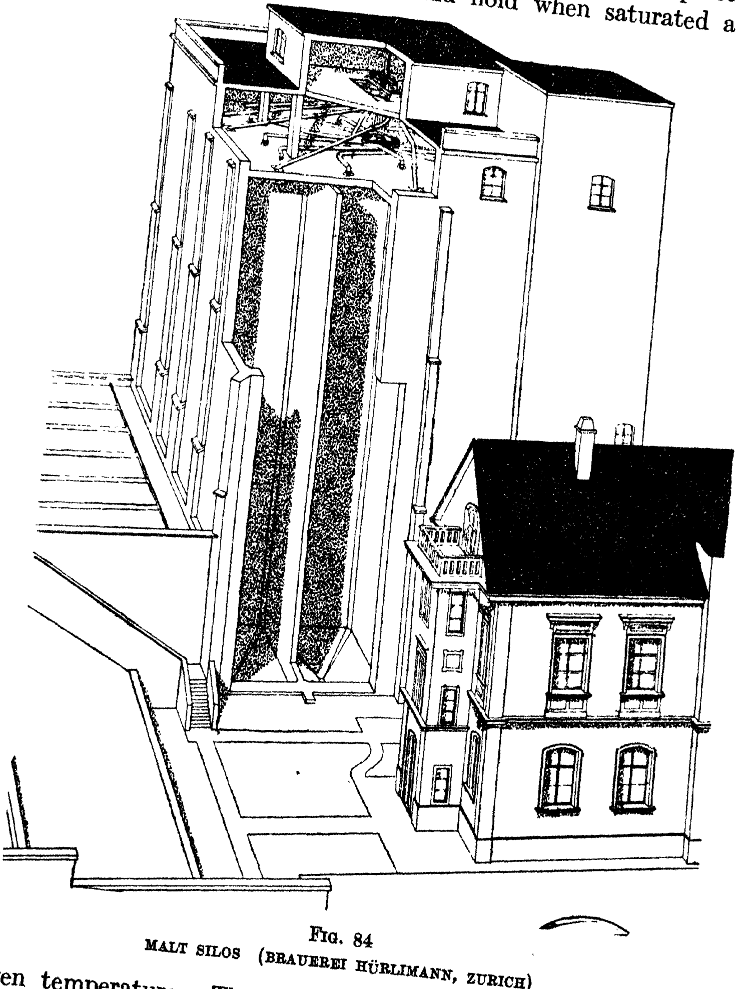


Fig. 84
MALT SILOS (BRAUEREI HÜRLIMANN, ZURICH)

TABLE 152.—WEIGHT OF AQUEOUS VAPOUR IN AIR SATURATED AT DIFFERENT TEMPERATURES AT NORMAL ATMOSPHERIC PRESSURE

Temperature	Grams water per Cubic Metre
32° F.	4·871
41° F.	6·795
50° F.	9·362
59° F.	12·746
68° F.	17·157
77° F.	22·843
86° F.	30·095

(388) Retorrification of Malt.

Hot grist mashing was introduced more than 50 years ago by Clinch[1] who raised the temperature of the grist approximately to that of the striking liquor by passing steam through pipes in the grist case. The advantages claimed were, in addition to removal of excess moisture, improved flavour and aroma in the beer and protection of the enzymes from the thermal shock caused by the high striking heat necessary with cold grist. It was contended that wort of improved composition was obtained, containing a greater proportion of simple and medium molecular protein derivatives for yeast nutrition and production of beers with greater palate fulness and better foaming properties. Retorrification has undoubted practical advantages of the kind originally claimed, but it is now usually applied to unground malt in a drum similar to that used for drying barley. These are made of 10 to 50 quarters capacity, the 50-quarter drums being about 8 ft. 6 inches in diameter by 15 feet in length. These require a motor of 2 h.p. for revolution and another of 5 h.p. for the fan. The drum consists of a mild steel cylinder, mounted on rollers, and revolves about once in ten minutes. Heated air from an anthracite furnace is drawn by a fan through a number of perforated tubes in the drum, thence through the malt and discharged into a dust room or collector.

The malt is screened before loading to the drum, where the heat treatment varies according to requirements, the air temperatures rising, for example, from 150° to 165° F., or from about 170° to 220° F., which, after 8 or 5 hours, respectively, would bring the temperature of the malts to about 140° or 180° F. The malt is usually ground in the evening and left in the grist case for mashing the next morning, its temperature falling by 20°–40° F. The moisture of the malt rises during the first hour or two, on account of condensation on the cooler corns of water carried by the heated air. Hence the necessity for 8 hours' treatment at 150° F., which does not materially reduce the diastatic activity of the malt.

(389) Preparation of the Grist.

The object of grinding or crushing is to bring the malt into such a state of division that the enzymes shall be able to act most efficiently on its starch, protein and other constituents during mashing. The extract obtained depends, in no small measure, on the manner in which this is carried out. It might appear that the most thorough conversion would be secured by reducing the malt to a fine flour but, in practice, this is far from the case. Reliance has to be placed on the bed of grains for filtration, whether this is in a mash tun or filter, and this filtering bed must be sufficiently porous to facilitate rapid sparging out of extract absorbed by the grains. This requirement is effectually opposed by grinding the malt to flour, some of which collects in the slots of the mash tun plates or passes through them, in the form of an imperfectly converted paste. The gain in extract, which might be obtained by fine grinding, must therefore be balanced against the necessary porosity, which is secured by even distribution of unbroken husks throughout the bed of grains in an infusion mash or by a stratification of the husks in the lower part of the grains in a decoction mash. It is necessary to find by experiment the most satisfactory degree of division for each kind of malt, for the method of mashing adopted and available plant. Friable malt may be ground more coarsely than steely, without loss of extract, while modern mash tun machinery can cope with more finely divided grist than older plant. A finer grist is also permissible with a mash filter than with a mash tun or lauter tun, but help from the husks is required, even with the filter. The pores of the cloths rapidly become clogged by an excess of flour.

Satisfactory grinding is judged by reduction of the interior of the corn to grits, with the minimum of flour and with husks as unbroken as possible. It depends on breaking the husks, longitudinally if possible, just sufficiently to detach from them the internal parts of the corns, leaving no adherent hard ends. The endosperms must then be broken to grits, no more than fine enough to allow of complete and rapid conversion. If the malt is steely, the grits must be smaller than with a friable malt and any hard ends must be most finely ground of all. A proportion of flour is inevitable, but it must be kept as low as possible. Success in this process is reflected by the extract and the ease with which it is obtained. Inefficient grinding may result in lack of brilliance in the worts or beer, as well as in lack of extract. This may be due to incomplete conversion of steely grits or of paste from the flour. The protein may also give rise to trouble if the malt is too finely ground and it is extracted in an incompletely digested form during sparging. Too fine grind, with resulting lack of buoyancy in the goods, may lead to a dead or flooded infusion mash, with very slow drainage, the sparge liquor collecting in pools or to a considerable depth all over the goods.

(390) Equipment of Mill Rooms.

The milling plant consists of screens for polishing or grading the malt, an automatic weighing machine, delivering direct to the mill, and efficient means for dealing with the dust from all parts of the plant. The whole is advisedly constructed on the tower principle, the ground malt being passed to a grist case commanding the mash tun, though Jacob's ladder conveyors are used in some cases to lift the ground malt to the grist case. The malt is delivered by mechanical or pneumatic conveyors to the screens. Fig. 85 represents an installation by Boby Ltd., comprising a rotary screen for removing culms and large substances and fitted with suction fan for dust disposal. The malt is delivered direct to an automatic weigher and thence to the mill, which commands the grist case. The installation also embodies a feeder for flaked maize. This consists of a hopper fitted with stirrers, delivering into a short screw conveyor which is operated by a ratchet wheel, so that the quantity required can be nicely adjusted to blend evenly with the malt.

In the majority of cases the grist case is fixed above the mash tun, and it may conveniently take the form of a steel silo with conical bottom. Several of these may be suspended or mounted on wheels to move as required to connect with a chute to the mash tun. These silos may be balanced for weighing the grist in them. An extra silo, with mill and grist case commanding a cooker, may, with advantage, be allotted to grits.

(391) Dust Disposal.

Special attention should be devoted to the efficient removal and disposal of dust produced in screens, mills and elevators, in order to prevent infection in the brewery by bacteria and spores which are invariably carried with it. All doors and communicating passages from the malt and mill rooms must be well fitted or screened and suitable ducts connected to every possible source of dust in the machinery. The dust is forced through these by suction or pressure to some form of dust collector or quencher.

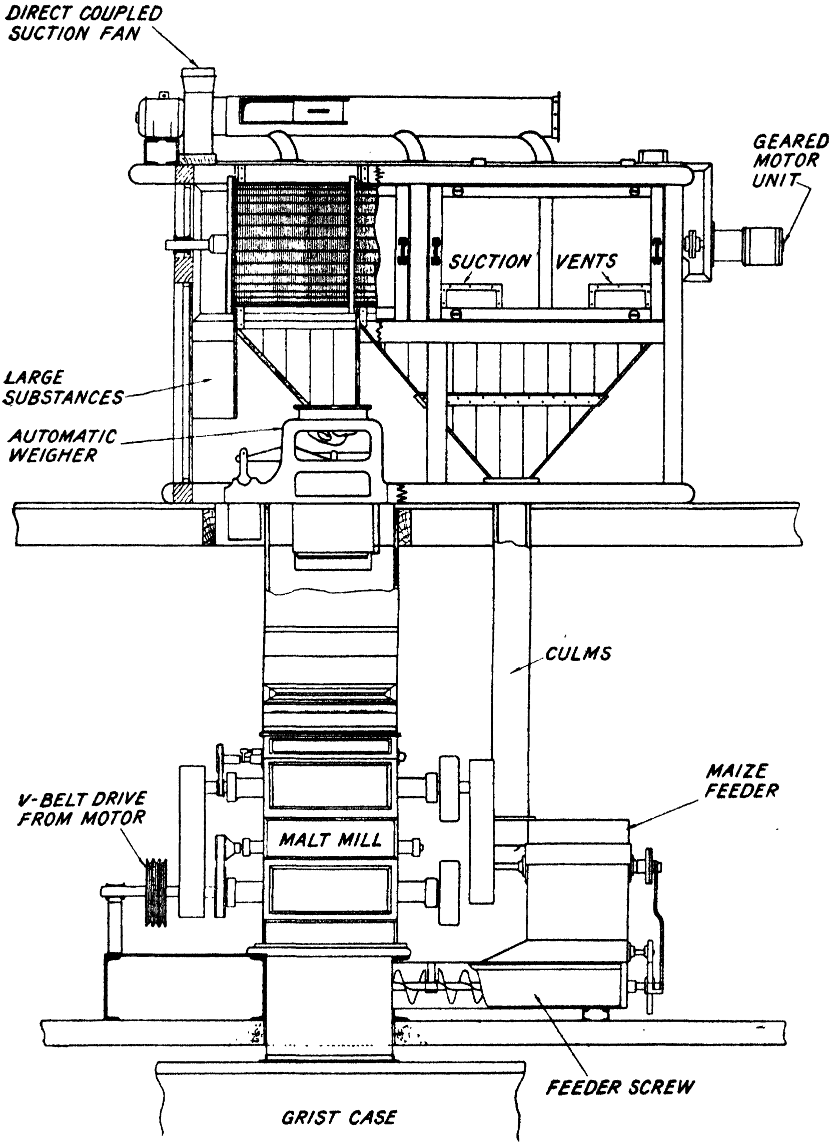


FIG. 85

SECTIONAL ELEVATION OF SCREEN AND MILL

A simple and most efficient arrangement consists of a powerful fan which aspirates the dust to a central point, from which it is blown to the bottom of a tank outside the building. The tank is divided in two chambers by a perforated partition, the upper containing two feet of coke, which is continuously sprinkled with water (Finzel[2]). The air on entering the lower chamber from the air duct loses its velocity on account of the increased area. The heavier particles of dust drop to the floor of the chamber, while the finer particles are carried up by the air current into the coke bed. The fine dust is retained by the moistened coke and washed therefrom by the liquor falling from the sparge, which also washes away the heavier particles from the lower chamber to a drain or other suitable means of disposal. The air discharged from the surface of the coke is free from dust.

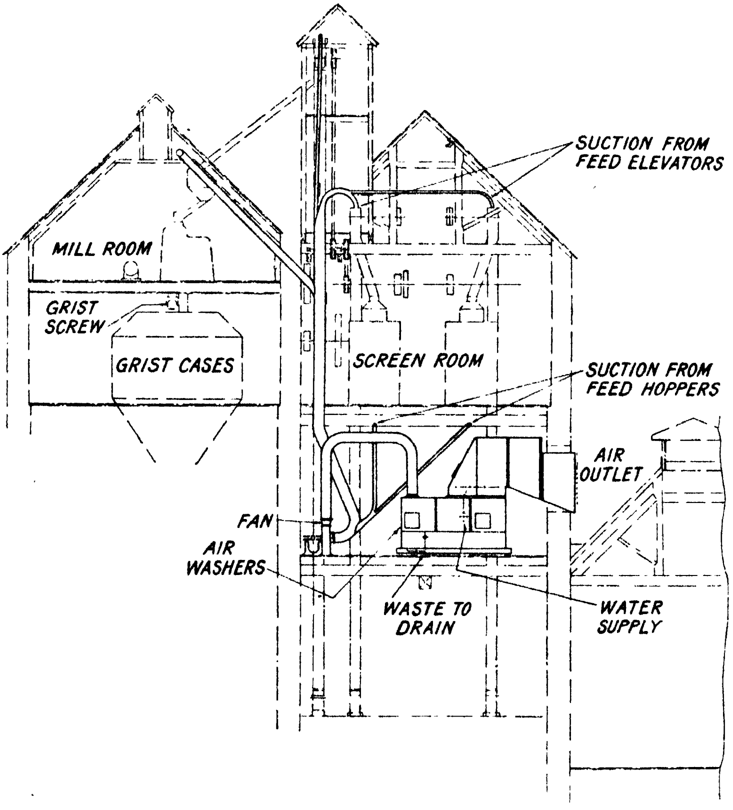
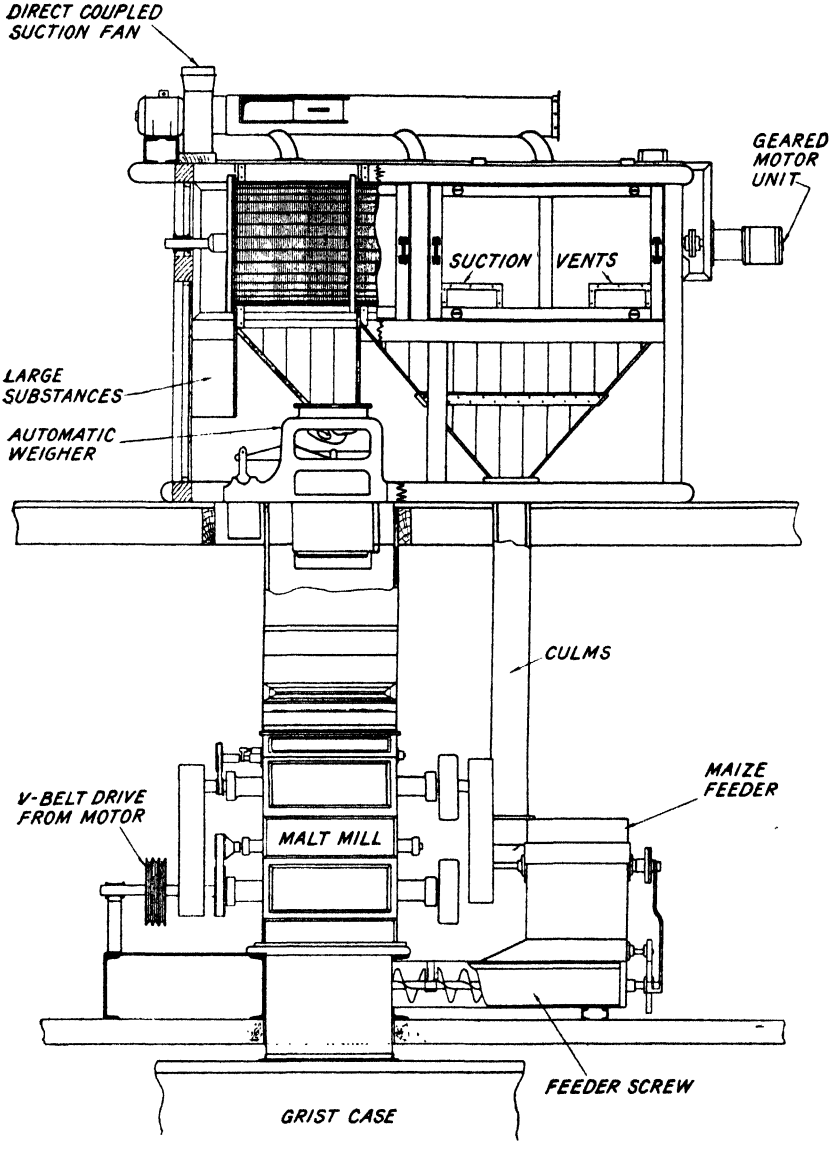


FIG. 86

DUST DISPOSAL PLANT IN MILL ROOMS

Among other systems are Cyclone dust extractors and tubular cloth dust collectors and air filters. The canvas sleeves are, however, not generally so efficient as more elaborate systems in which the dust is water-quenched. A lay-out of the latter type by Boby is shown in Fig. 86. This collects dust from the rotary screens, head of malt elevators and from the hopper feeding to the mills. The fans discharge to a tank of stout, galvanised, sheet metal, internally fitted with a series of baffle plates and a large number of high-pressure water spray nozzles. A series of



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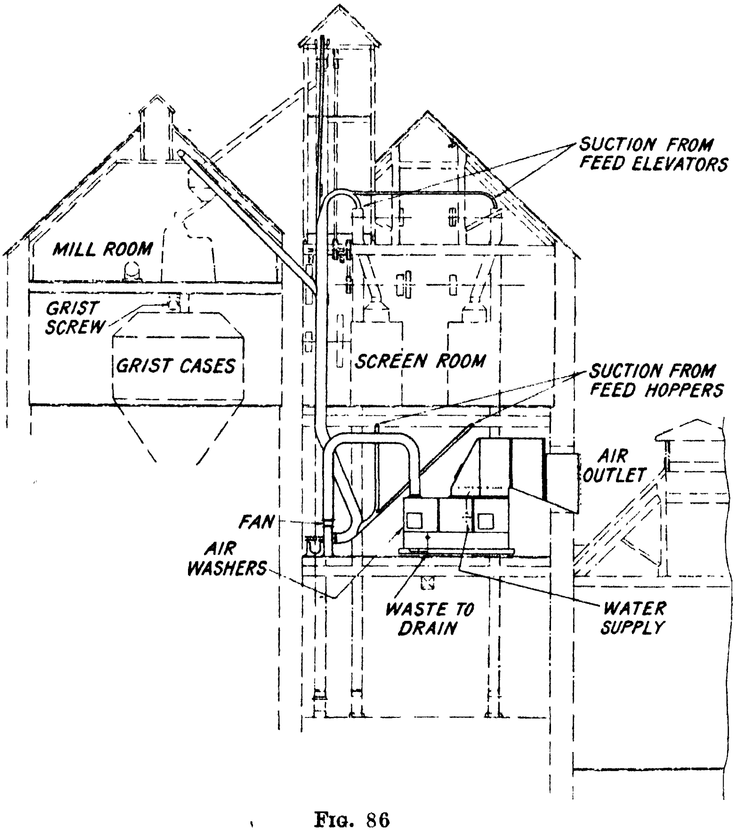

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(392) Design of Mills.

The mill should be of as simple design as possible, consistent with efficiency under the conditions of operation, but it does not pay to sacrifice extract to original cost. The most suitable design depends on the average type of malt and mashing system.

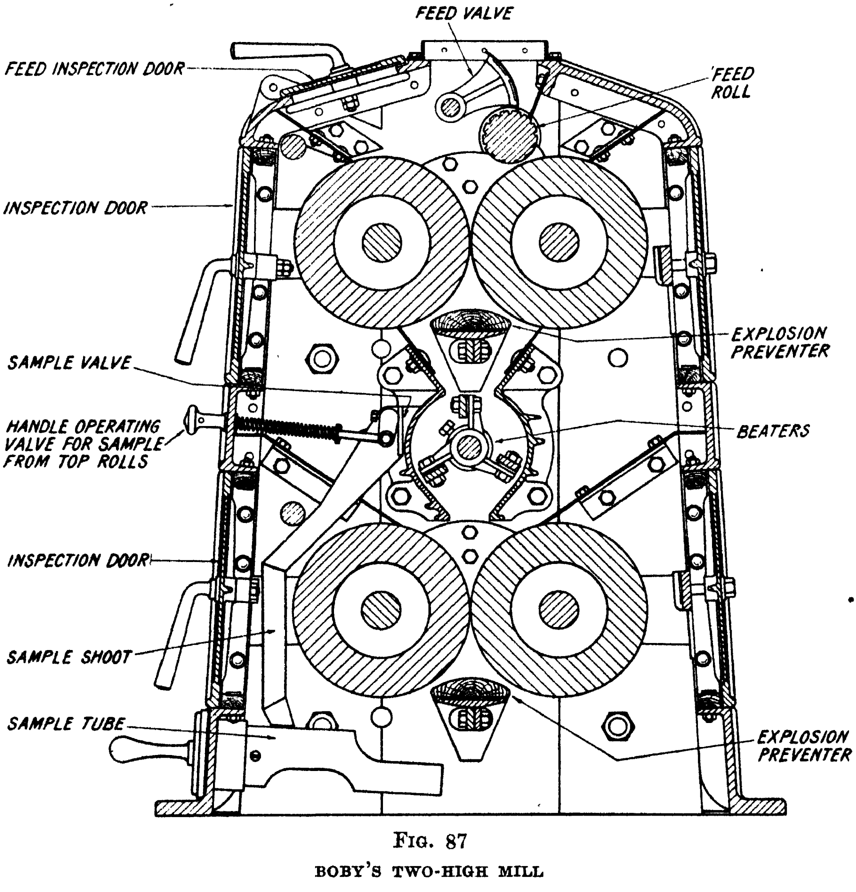

Fig. 87

BOBY'S TWO-HIGH MILL

If the malt is tender and of even size, without hard ends, a simple mill will do all that is required, but mills with one pair of rolls are now seldom used. Two or three pairs of rolls deal more efficiently with malt of varying or average quality. The simplest of these has four rollers, one pair vertically above the other, as shown in the diagram of the Two-High mill, Fig. 87, which is generally erected with screen and automatic weigher as shown in Fig. 85.

The malt, after passing over magnets to abstract pieces of iron, is distributed by a small feed roll, evenly over the first pair of crushing rolls. These are set only to crack and open the corns, which must then be emptied of their contents as completely as possible to ensure that the husks collapse without being unduly broken by the second pair of rolls, which should reduce the large grits without forming flour, Separation of husks and grits is accomplished, in the Two-High mill, by means of a revolving beater, working in a cast iron casing. This whirls the crushed corns against the side and releases the grits. Any hard ends remaining in the husks are crushed in the lower rolls with the grits. The upper rolls are driven at about 200 revolutions per minute and the lower at about 300. The possibility of explosions arising, from pieces of stone which have not been separated in the screens, is minimised by shelves placed beneath the rolls. These ensure that the spaces immediately below the rolls, as well as those above them, are full of grist which prevents the passage of sparks to dust in the mill or elevators. Malt dust suspended in air is highly explosive, and it is customary to

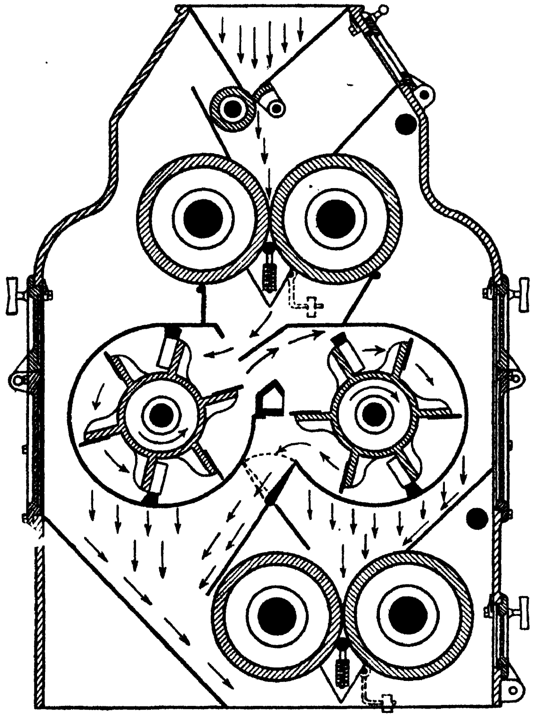

FIG. 88

DIAGRAM OF PORTEUS FOUR-ROLLER MILL

arrange for some vent or lightly covered aperture in elevators, etc., so that the blast can pass without material damage if an explosion should occur.

In order to deal to the best advantage with the larger and smaller corns in mills of this type, the malt is graded on screens and fed to two mills with rolls suitably adjusted. Machines are available for blending the grist of two- and six-rowed malts after separate grinding, but it is more customary to grind and run the two-rowed malt to the grist case first, following it with the six-rowed malt, for which the rolls are readjusted, if necessary. The upper layer of husky malt runs down first from the grist case, through the centre of the grist, and forms the larger portion of the lower strata of goods in the mash tun.

In the Porteus mill, shown in section in Fig. 88, cylindrical screens fitted with mechanism to detach the hard ends from the husks are used. The fine grits produced in the first pair of rolls pass from the left-hand cylinder direct to the grist case, meeting the husks from which hard ends have been detached in that on the right. The coarse grits pass from the latter to the second pair of rolls. Two of these units built together constitute the eight-roller mill which is fitted with a malt grader to serve the two divisions.

Shaking screens are fitted in other mills between the pairs of rolls to separate the crushed malt from the upper pair in two or more grades and pass that which requires further crushing to the lower pair. The Seck mill is of this type and may contain either two or three pairs of rolls. The first pair are slightly grooved to grip the corns and crush them slightly before passing to the screen. In the two-roller mill the fine grits are delivered directly to the grist case, while the husks with adhering hard ends go to the lower pair of rolls, which are set to crush the hard ends without damaging the husks. On leaving the second pair of rolls, the husks, etc., mix intimately with the fine grits from the top rolls as they enter the grist case. In the six-roller mill shown in Fig. 89, the large steely grits are shaken from the husks and passed to a third pair of rolls, which reduces them further, while the husks are delivered without further damage. Mills of this type, surmounted by cylindrical screens in cast iron casings, are illustrated in Fig. 83.

In lager breweries the five- or six-roller mills are preferred, as they are more efficient for grinding less fully modified malts than those with two pairs, but they show little if any advantage with the more friable malts generally used in top fermentation breweries. The best grist for a mash filter is obtained from a mill in which the top pair of rolls is fluted, while a mill with a single pair of rolls is used for grits. Whichever type of mill is adopted, it is necessary to sample the grist from time to time, preferably by means of a sampling tray extending the whole width of the rolls. With this it is possible to determine by sight and feel whether the roller separation is regular from end to end and the grind is correctly adjusted. Other samples are sieved to control the proportions of husk, grits and flour. The mechanism for roller adjustment is very delicate and accurate in modern mills and, after setting, can usually be locked. Wear on the rolls is bound to occur, but refacing should only be required very infrequently.

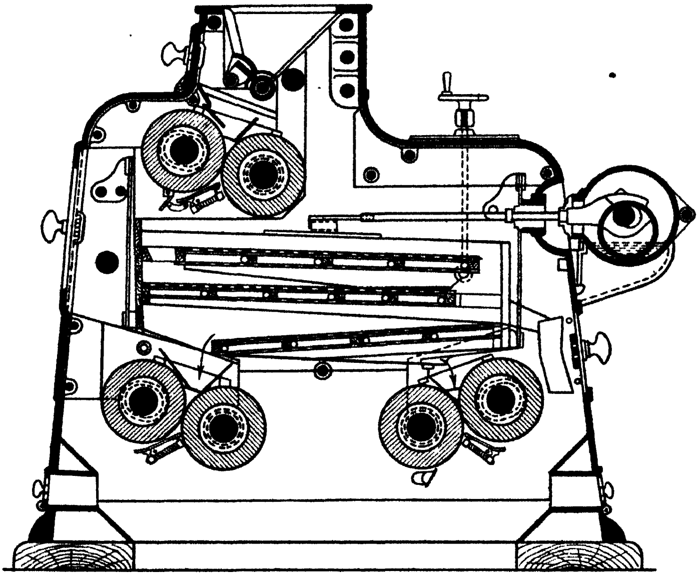

Fig. 89

SECTION OF WORSSAM'S SECK TYPE UNIVERSAL MILL

(393) Grist Fractions.

Highest possible extract is secured, so far as milling operations can effect it, by adjustment of the rolls to get a balance of husk, grits and flour appropriate to the malt and mashing plant and by even mixture of the different fractions of the grist. The following proportions by weight may be taken as a rough guide for English malts of good quality and infusion mashing, the separation being made in a triple box screen (Table 153).

With the more elaborate *Pfungstadt-Plansichter*, adopted as standard screen for the Congress method of malt analysis and used in some lager breweries, the separations in Table 154 might be obtained with the five screens and the less fully modified malts used for the decoction mash.

TABLE 153.—GRIST FOR INFUSION MASH
(English malt, 42 lb. per bushel)

Screen	Fraction	Weight %	Weight per bushel
10 squares per in. ..	Husk	32	17 lb.
16 " " ..	Large grits ..	23	31 "
Silk	Small grits and coarse flour ..	39	38 "
Bottom	Fine flour ..	6	36 "
Total grist	——	100	36 "

TABLE 154.—SCREENING OF LAGER GRISTS. PFUNGSTADT SCREEN

Screen mesh mm.	Coarse grist	Fine grist
1·27	15	10
1·01	5	2
0·547	25	15
0 253	30	43
0·152	10	10
Bottom	15	20

The fractions obtained in an ordinary four-roller mill give entirely different analyses, as shown in Table 155 (Briant and Harman[3]).

TABLE 155.—ANALYSIS OF GRIST FRACTIONS (BRIANT AND HARMAN)

	Flour	Grits	Fine husk	Coarse husk	Total malt
Extract per 336 lb.	115·8	109·4	70·6	68·9	102·5
Moisture %	5·0	2·6	4·5	2·0	—
Colour, 20 lb. boiled wort ..	7	11	15	14	—
Diastatic activity, Lintner°	15	24	23	14	—
Cold water extract % ..	15·0	18·6	19·9	16·0	—
N × 6·25, before boiling ..	2·6	3·7	7·2	6·2	4·0
N × 6·25, after boiling ..	2·5	3·6	7·1	5·9	3·8
$[\alpha]_D$ of dry solids	134·5	124·0	100·5	103·5	123·0

The flour is not necessarily the best modified part of the endosperm and may have resulted from fine grinding of the hard ends of the corns. Microscopical examination and speed of conversion indicate that the flour may be more resistant than the grits to conversion. Flour mashed at 150° F. was not completely converted in 2 hours, but the addition of 10% of highly diastatic malt reduced this time to 1 hour. The saccharification of large grits and husks, not passing a sieve with 100 mesh to the square inch, was extremely slow and not appreciably affected by diastatic malt extract. On the other hand, the grits and husk, finely ground to pass a 1000-mesh sieve, ceased to give a colour with iodine in 35 minutes and there was no increase in the rate of saccharification after addition of highly diastatic malt extract. Briant and Harman recorded very different characteristics in beers obtained experimentally from the different fractions. That from the flour was very soft and somewhat characterless, yet full drinking. It retained its head well, but did not prove easy of attenuation. The husks gave a beer of thin, almost acid flavour and evidenced symptoms of unsoundness on storage. By far the best balanced beer was obtained from the grits.

MATERIALS FOR THE BREW

(394) Selection of Materials.

Lintner's well-known dictum that " malt is the soul of beer " is regarded as axiomatic in brewing. It may be difficult to assess the relative importance of malt, adjuncts, liquor, yeast and brewing methods in determining the character, quality and stability of beer, but no care expended on selection of the primary raw material can be considered too great. The principles governing the valuation of malt and its selection for different purposes were described in Volume I. The actual choice of malts for the grist is a very personal matter, influenced by such local considerations as preferences for certain types of beer or traditional brewing methods and available plant, and is, not infrequently, restricted to local supplies for a considerable part of the requirements. Certain general considerations may, however, be applied to the selection of malts for different kinds of beer, and these will be illustrated by reference to the malt analyses given in Chapters XII and XIII. Malt is primarily a source of extract, but the yield plays little part in the quality of beer. Other characters on which information can be gained by inspection, bite and taste, together with the ordinary methods of analysis, are of much greater importance. Physical examination affords information on variety, friability, flavour, damage, mixture of other seeds and on the possibility of deterioration through storage with an excessive moisture content. Analysis provides more precise evidence of the effects of germination and curing. Selection must be guided by both, and based on appreciation of the significance of all the available data. The most useful analytical results include the total nitrogen and permanently soluble nitrogen, considered in relation with the cold water extract, the colour and its connection with flavour and enzymic activity. Of these, the relation between the permanently soluble and total nitrogen is of particular importance, in view of its implications in respect of modification and the malting changes in other barley constituents, on which the quality and stability of beer depend.

Examples of grists for different kinds of beers are given in the following Sections. They could be varied in many ways, and would have to be altered in accordance with the available malts. They must, consequently, only be regarded as illustrative of how the malts described in Chapters XII and XIII could be utilised to brew about 100 barrels of beer of the types indicated. Sugars, added in the copper, are given in the Tables to complete the materials for the brews. The quantities are stated in quarters, 2 cwt. of sugar being taken as equivalent to 336 lb. of malt, flakes or grits. The percentages are calculated on the total extract yield.

The special malts described in Chapter XIII can all be used with advantage in appropriate circumstances. Their value, and the most suitable proportions to use in particular cases, should be ascertained by trial. Examples of their uses have been included in one or other of the grists. Enzymic malt finds application at the rate of between 3 and 5% in a large proportion of grists to increase the acidity of the mash (Section 458), or on account of its exceptionally high bios content (Section 682). Diamber (Section 217) gives a very full flavour and aroma, yet it retains a normal diastatic activity ; 5 or 10% can be used in mild or dark ales to increase fulness or flavour. A similar quantity of well-modified wheat malt (Section 218, No. 3), adds fulness and head retention, and is also useful for yeast nutrition. Diastatic malt extracts (Section 220) are used in many breweries, at the rate of about 1 cwt. per 12 quarters of malt, and non-diastatic extract syrups are sometimes added in the copper. The roasted malts, black, brown, amber and crystal, or roasted barley, are used in ales as well as stouts to give colour and add their characteristic flavours.

(395) Pale Ale Grists.

Pale ales have to comply with higher standards of stability and brilliance than mild ales, while they lack certain constituents of dark beers that hinder the formation of protein haze, and contain a greater quantity of the hop tannins which participate in its production (Sections 496 and 709). This means that the malts are subject to more stringent requirements, and must be made from barleys of the highest classes, sound, thoroughly matured and undamaged. The typical pale ale malts are of fine appearance, completely friable, well modified and soundly cured, however pale they may be. The malts selected for pale ales from Chapter XII are as follows, the numbers refer to the analyses given in that Chapter.

	Extract lb.	Colour	Nitrogen %	P.S.N. % of N.
1 English	100·5	4·5	1·34	38·0
4 "	98·5	6·0	1·47	39·2
6 "	99·0	6·0	1·40	38·6
13 "	97·5	4·0	1·55	36·0
14 Moravian (or equivalent English)	98·9	6·5	1·52	36·9
19 Californian	91·9	6·0	1·61	30·1
20 "	94·0	4·0	1·51	30·2
26 Syrian (or equivalent six-rowed malt)	90·6	5·5	1·43	33·6

The Spratt-Archer malt, No. 1 (Section 195), is a good selection for an all-malt pale ale of 1050–1055 original gravity. Its low nitrogen content is characteristic of high class English malts, but might provide insufficient yeast nutrients in low gravity worts, particularly if adjuncts which yield no nitrogen are used with it (Sections 205–206). Malts giving analyses comparable with Nos. 4, 13 and 14 are to be preferred when a moderate proportion of sugar, maize or rice is used. Malts intended for non-deposit bottled beers must be fully modified, in order to avoid early haze formation, though this may be at the expense of some depreciation in fulness and head retention. The analyses of Nos. 1, 4 and 14, giving 38·0, 39·2 and 36·9 for P.S.N. as % of total nitrogen, are appropriate. The total nitrogen contents of the malts, 1·34%, 1·47% and 1·52%, correspond with their suitability for use with increasing proportions of adjuncts. The modification of No. 13 is good for an English malt containing 1·55% of nitrogen, but is about as low as it is desirable to go for filtered bottled beers. The P.S.N. % of total nitrogen may be slightly lower in malts intended for draught pale ales.

It is advisable to set a limit of about 3% to the moisture content of malts intended for infusion mash brewing. The colour of the malt usually corresponds fairly well with its flavour and should, in most cases, be as high as the colour of the beer will permit. It must be low, between 3·5 and 4·5, for all-malt pale ales of high gravity. It can be increased to 6 or 7 for lighter gravity, pale beers, particularly if flakes or grits are used. Diastatic activities around 28°–35° Lintner are adequate. Regularity of colour and other properties is important. It is generally found to be better secured by blending selected malts than by reliance on the uniformity of one large bulk.

It is usual to include a proportion of six-rowed Californian, Smyrna or other malt of similar type, with the British malts in the grists. 50% was formerly common, but this has now been reduced to between 10 and 20% in most cases. An index of modification of 30–32 is satisfactory with these malts. Nos. 19, 20 and 26 (Sections 200 and 201), are examples of suitable types. Some brewers might consider No. 19 as of mild ale grade, but others would prefer it to the finer-skinned Atlas malts, such as No. 20, for blending purposes. No. 25 is definitely under-modified, and No. 21 is insufficiently modified for non-deposit bottled beer, though probably quite satisfactory for draught beers.

TABLE 156.—GRISTS FOR PALE ALES

Malt No. and Adjuncts	Quarters	% of total extract	Brewers' pounds		Degrees gravity		Barrels and gravity
			Extract of malt, etc.	Extract yield	Extract of malt, etc.	Extract yield	
All-malt pale ale at 1,055							
1	16	85·1	100·5	1,608·0	279·2	4,467·2	95 brls.
20	3	14·9	94·0	282·0	261·1	783·3	19·9 lb. or
				1,890·0		5,250·5	1,055·3
Pale ale 1,050 *with sugar and maize*							
6	7	37·7	99·0	693·0	275·0	1,925·0	
14	6	32·3	98·9	593·4	274·7	1,648·2	102 brls.
26	3	14·8	90·6	271·8	251·7	755·1	18 lb.
Maize	1	5·5	100·0	100·0	277·8	277·8	or
Malt extract	½	1·9	70·0	35·0	194·4	97·2	1,050·0
Sugar	2	7·8	72·0	144·0	200·0	400·0	
				1,837·2		5,103·3	
Light pale ale for bottling, 1,040							
4	4	27·7	98·5	394·0	273·6	1,094·4	
13	4	27·4	97·5	390·0	270·8	1,083·2	100 brls.
19	3	19·4	91·9	275·7	255·3	765·9	14·2 lb.
Enzymic	½	3·3	94·0	47·0	261·1	130·5	or
Maize	1	7·0	100·0	100·0	277·8	277·8	1,039·5
Sugar	3	15·2	72·0	216·0	200·0	600·0	
				1,422·7		3,951·8	

The use of flaked maize, rice or grits and sugar depends on the character of the beer. Sugars add sweetness and, like flakes, reduce the tendency to early haze formation in filtered beers, particularly if the malt is at all under-modified. The sugars must be pale in colour and carefully selected in accordance with the flavour required in the beer. No. 1 and No. 2 inverts and other sugars of somewhat similar character are suitable. The percentage of adjuncts ~~used varies with~~ the type of beer, and may provide 10–25% of the total extract.

(396) Grists for Mild and Dark Ales.

TABLE 157.—GRISTS FOR MILD AND DARK ALES

Malt No. and Adjuncts	Quarters	% of total extract	Brewers' pounds		Degrees gravity		Barrels and gravity
			Extract of malt, etc.	Extract yield	Extract of malt, etc.	Extract yield	
Strong ale, 1,080							
3	10	34·3	98·4	984·0	273·3	2,733·0	100 brls.
8	10	33·9	97·2	972·0	270·0	2,700·0	
19	4	12·8	91·9	367·6	255·3	1,021·2	28·6 lb.
Crystal ..	1	3·2	90·5	90·5	251·4	251·4	or
Amber ..	1	3·2	90·5	90·5	251·4	251·4	1,079·6
Sugar	5	12·6	72·0	360·0	200·0	1,000·0	
				2,864·6		7,957·0	
Mild ale, 1,045							
5	4	24·6	100·6	402·4	279·4	1,117·6	100 brls.
9	6	35·5	96·8	580·8	268·9	1,613·4	
19	3	16·8	91·9	275·7	255·3	765·9	16·4 lb.
Crystal ..	½	2·8	90·5	45·2	251·4	125·7	or
Brown ..	½	2·8	90·5	45·2	251·4	125·7	1,045·5
Sugar.. ..	4	17·5	72·0	288·0	200·0	800·0	
				1,637·3		4,548·3	
Mild ale, 1,040							
7	3	20·5	99·4	298·2	276·1	828·3	100 brls.
9	4	26·7	96·8	387·2	268·9	1,075·6	
24	3	19·3	93·1	279·3	258·6	775·8	14·5 lb.
Diamber ..	1	6·5	95·0	95·0	263·9	263·9	or
Wheat malt ..	1	7·1	102·6	102·6	285·0	285·0	1,040·3
Sugar.. ..	4	19·9	72·0	288·0	200·0	800·0	
				1,450·3		4,028·6	

The malts are usually of rather lower grade than those selected for pale ales. They are cured to a colour of 6 to 9, to give full flavour, and it is not usually necessary to require such full modification. P.S.N. between 34 and 36% of the total nitrogen is adequate, the lower figure giving the necessary fulness and foam stability in draught beers, with less liability to haze in bottled beers than would be the case in pale ales. Crystal malt and amber malt, particularly oak-dried amber, are frequently used on account of their colour and flavour. A larger percentage of sugar is generally added in the copper than for pale ales, and darker sugars, with more luscious flavours, are selected. No. 3 invert, good quality raw cane sugar and proprietary mixed sugars of various descriptions, with a little caramel, are suitable. The grists in Table 157 have been made up from the following malts :

	Extract lb.	Colour	Nitrogen %	P.S.N. % of N.
3 English	98·4	5·5	1·46	34·6
5 Scotch	100·6	6·5	1·32	36·9
7 English	99·4	7·0	1·47	33·3
8 ,, ...	97·2	8·0	1·47	34·9
9 ,, ...	96·8	7·5	1·60	35·1
19 Californian	91·9	6·0	1·62	30·1
24 Chilean (or similar six-rowed malts)	93·1	6·0	1·52	31·9

(397) Grists for Stouts.

There are ~~a number of distinct types of stout and por~~ter for which different blends of material are used. On the one hand, are the stouts brewed from malt only or from malt and roasted barley. On the other, are the sweeter stouts, for which a fairly high percentage of sugar is employed. The basis of the grists is a mixture of pale malt, not too fully modified, but with a moderate diastatic activity, and either roasted malt or roasted barley to give the requisite colour and flavour. Roasted barley gives a drier flavour than roasted malt and is preferred by many. Crystal and amber malts are commonly blended with these in the sweeter stouts. A proportion of six-rowed malt and of maize is also frequently used, as in mild ales. A limited percentage of oat malt or oat flakes is included in the grists for oat malt and oatmeal stouts. Milk stouts generally derive their name from the lactose or milk sugar added, with cane or other fermentable sugar, in the form of primings. The copper sugars are generally full flavoured and dark coloured. Good raw cane sugar, No. 3 invert and various mixed sugars are suitable, a sweet caramel being added if it is desired to increase the colour without the special flavour and aroma of roasted malt or barley. The grist in Table 158 is an example of what might be used in a full, sweet stout, with the malts listed in the last Section.

The ~~colours of~~ stouts usually vary from about 250 to 400, in the 1-inch Tintometer cell. That of roasted malts is given in analyses for 10% worts in the 1-inch cell ; 1 quarter of malt per 100 barrels is approximately 1% (336 lb. in 36,000 lb. = 0·93%). The number of quarters required for each 100 barrels can thus be calculated roughly from

$$\text{Number of quarters} = \frac{\text{Stout colour} \times 10}{\text{Malt colour}}$$

An approximation for the quantity of caramel required, in addition to or in replacement of malt, can be obtained from Table 75, Section 212. The colour of the stronger stout produced from the grist in Table 158 might thus be expected to be about 430, and that of the stout at 1045·7 about 330, allowing about 20 for the colour given by the pale malt, hops, boiling, etc.

Table 158.—Grist for Sweet Stout

Malt No. and Adjuncts	Quarters	% of total extract	Brewers' pounds: Extract of malt, etc.	Brewers' pounds: Extract yield	Degrees gravity: Extract of malt, etc.	Degrees gravity: Extract yield	Barrels and gravity
3	6	27·6	98·4	590·4	273·3	1,639·8	100 brls.
7	5	23·2	99·4	497·0	276·1	1,380·5	21·4 lb.
Roasted malt or barley ..	3	12·1	86·0	258·0	238·9	716·7	1,059·4 or
Crystal ..	2	8·4	90·5	181·0	251·4	502·8	130 brls
Amber ..	2	8·5	90·5	181·0	251·4	502·8	16·5 lb.
Sugar.. ..	6	20·2	72·0	432·0	200	1,200·0	1,045·7
				2,139·4		5,942·6	

(398) Grists for Lager Beers.

Lager malts differ rather widely from pale and mild ale malts, as is shown by the analyses in Sections 202–204. Those used in Europe differ mainly on account of the malting process, though a higher nitrogen content is characteristic, about 1·6% being regarded as most suitable for many types of beer. Pale lager malts are much more lightly cured than pale ale malts. The dark malts used for beer of Munich type have a distinctly aromatic character. The degree of modification is generally definitely lower than that of English malts. The relation between modification and the various decoction mashing systems is dealt with in Chapter XXIV, from which it will be gathered that single- or double-mash processes are most appropriate for more fully modified malts, while the three-mash process deals more satisfactorily with malts of lower modification. Selection of malt must therefore be governed by the mashing process as well as by the beer type, Pilsen, Dortmund, Munich, etc. Adjuncts are not used in Germany, but 10–25% of rice or maize grits are employed in most other countries, with malts similar to Nos. 34 and 35 in Section 203. Sugar is used to a much smaller extent.

The malts used in American bottom fermentation beers differ as much in the type of barley malted as in the malting process. Their characteristics are described in Section 202, Nos. 28 to 31 represent samples from one of the largest American maltsters. They are used with 25–40% or more of rice or maize grits. Pale sugars are also widely employed in the copper, corn syrups (Section 233) being particularly applicable.

(399) Summary.

Selection of malt and other materials must be based on experience of the effects on beer character, under existing brewing conditions, of variations in the type, quality and composition of the malt, and of the influence of different kinds and proportions of unmalted cereals and sugars. The malt is of first importance, and skilled judgment of its physical characters must be supplemented by appreciation of the meaning of available analytical determinations. It does not do to lay undue stress on extract, as this varies inversely with nitrogen content in any particular type of malt. Consideration must be given to the presence of a sufficient quantity of nitrogenous yeast nutrients, and the reduction in these caused by substitution of unmalted cereals and sugar. It is, unfortunately, impossible to specify the minimum nitrogen requirements of yeast, but it is clearly desirable to employ low proportions of adjuncts with high extract-yielding malts. It is practically impossible to find sound, matured British barleys with a sufficiently high nitrogen content to permit of the use of the high proportion of grits commonly employed in America with Manchuria type malts, containing 2% of nitrogen. A good rule is to select malts with 1·5–1·6% of nitrogen for use with 20–25% of adjuncts. Alternatively, the percentage of the latter should be determined by the nitrogen content of the malts.

A second consideration of great importance in the selection of malts, is the extent of growth or modification. Requirements differ rather widely according to the type of beer produced and the method of brewing (Volume I, Section 182). It is possible to make a fairly reliable selection, from this point of view, by physical tests, but it is preferable to rely on some " index of modification " based on analysis. The simplest and most generally applicable test available at present appears to depend on determination of the relation between the permanently soluble nitrogen and total nitrogen (Sections 184 and 188). A good understanding of the implications of the nitrogen content of malt, and the permanently soluble nitrogen produced in the laboratory extract is most helpful in making up the grists. Full modification is essential for the production of non-deposit beers, with which lasting brilliance is required, while shorter growth may be preferable for draught ales and stouts, with which fulness and head retention are more important.

Consideration must also be given to the flavour of the malt and its colour, to the desirability of using a proportion of dark or other malts of special properties, and to the influence of unmalted cereals or sugars on beer flavour. Flakes and grits, while tending to a drier flavour, add to the colloidal stability of beer. Sugars leave a residual sweet flavour, even when completely fermented. Finally the quantities of the selected materials are adjusted according to the total extract required, and the percentage of that extract which each must provide. Although the extract of malt must be regarded as of less importance than its suitability for the type of beer required and the process adopted, it is necessary to secure all that it can yield. This depends primarily on the way it is crushed and, for this reason, every care must be taken to make the best use of the available milling machinery. Loss of extract from defective mills may very soon outweigh any reasonable expenditure on more efficient types, but that expenditure would be of little value unless the machines were properly used.

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CHAPTER XXIII

MASHING

(400) The Mashing Process.

Mashing is the process by which the ground malt or malt with a proportion of unmalted cereals is mixed with water and extracted to obtain the fermentable liquid known as wort or sweet wort, to distinguish it from the hopped wort which it becomes after boiling with hops. Before the introduction of thermometers in breweries, it was customary to let down the requisite quantity of nearly boiling liquor into the mash tun and, after it had cooled sufficiently for the steam to pass away and allow the brewer to see his face reflected in its surface, add the grist from sacks and thoroughly mix with rakes or mashing oars, as shown in Fig. 2, Vol. I. After this, nothing more was required until the wort was drawn off from the goods. This very crude method has been developed into the infusion mashing system, generally used in top fermentation breweries. Extraction of the ready formed soluble constituents of malt could be effected by cold liquor, but it is necessary to mash at temperatures around 145°–155° F., in order to facilitate enzymic conversion of the insoluble starch, protein and other substances, which contribute about three-quarters of the total extract. Practically all the starch of the malt is in this way converted into maltose and dextrins, but only about one-third to two-fifths of the nitrogen of malt is extracted in the form of soluble proteins or protein degradation products. The proportion of the hemicelluloses and other structural constituents of malt extracted in the form of soluble pentosans is even less, while about one-third of the inorganic constituents of malt is found in the wort.

The whole series of chemical and physical changes that occur during malting and mashing may be regarded as a continuous process of simplification, resulting in the production of a colloidal system of highly dispersed carbohydrate and nitrogenous substances in water, with simpler derivatives of the same groups and mineral substances in true solution. The transformations initiated during malting are continued in the mash tun at temperatures favourable to the activity of the enzymes concerned, until some 70% of the solid matter of the barley or 80% of that of the malt is extracted. The diagram in Fig. 90 (Russell and Bishop[1]) shows in a simplified and approximate manner the relative quantities of the more important groups of products obtained from two-rowed barleys, containing different percentages of nitrogen. As the nitrogen content of the barley increases, the reducing sugar and dextrin in the wort decrease, while its nitrogen content and the quantity of insoluble material or grains increase.

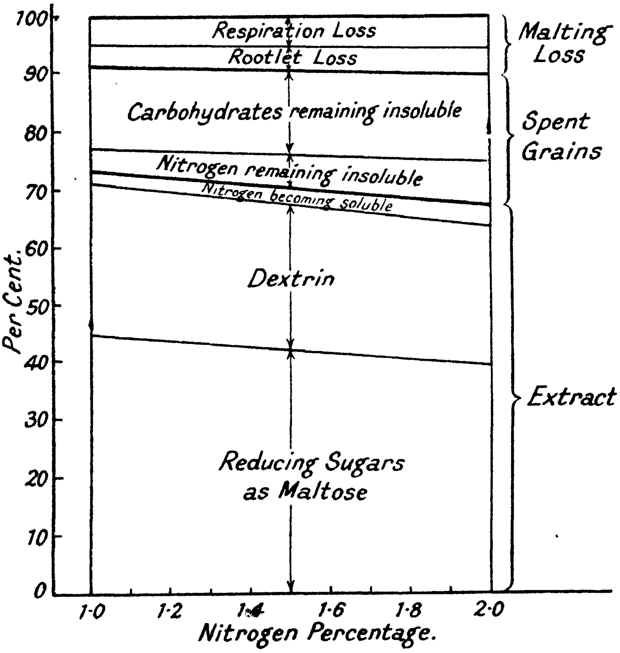

FIG. 90

DIAGRAMMATIC REPRESENTATION OF THE RELATIONSHIP BETWEEN THE COMPOSITION OF BARLEY AND WORT

This diagram suggests that the solid matter of wort consists mainly of fermentable sugars and unfermentable dextrins and that its nitrogenous constituents are comparatively insignificant in quantity. Despite this, they play a very important part in determining the quality of wort and beer. They may, however, be ignored for the present, while attention is directed to the starch-conversion products. Earlier investigators concerned themselves almost entirely with these, and devised methods of analysis, based on determination of the optical activity and reducing power of the wort, whereby they thought it possible to ascertain the relative quantities of dextrin and maltose in pure starch conversions, or to obtain an idea of the composition of wort and of its fermentability. These methods of analysis still form the basis of the analytical control of mashing, though, as will be explained in Chapter XXVII, such a simple conception of the composition of wort is entirely out of the question. For the present purpose it will, however, serve as a practical guide and it may be assumed that higher or lower mashing temperatures produce a smaller or greater proportion of maltose in the wort and that a greater proportion of dextrins is formed as the temperature is increased. Variation of the temperature of the mashing liquor is the most important of the different measures the brewer can take to control the composition of wort. By lowering it, within the normal range, he obtains a more saccharine and fermentable wort, and by raising it, a more dextrinous or less fermentable wort.

(401) Mashing Systems.

Mashing processes are subject to wide variation in detail in accordance with the nature of the grist and the type of beer required, but they will be described under three headings. The infusion mash, the decoction mash, and the double mash systems. The first consists in extraction of the malt by infusion only, while a portion of the mash is boiled in the decoction system. The third, designed for use with grits, involves gelatinisation of the latter by boiling with a portion of the malt and conversion of the gelatinised starch by mixing with a malt mash. The three systems are appropriate to different types of malt and yield worts of rather different composition. The maximum extract obtainable from the materials can be secured by any of them, provided the malt is appropriate to the process adopted and the latter is carried out in well designed plant. Modern improvements in mashing plant have been designed to facilitate and improve the mixing of the grist and liquor, to control the temperatures more perfectly and to expedite or increase the efficiency of the final removal of the worts from the grains.

The infusion mash is used with fully modified and friable malts in the production of top fermentation ales. The decoction system is better suited to deal with less fully modified malts and is that generally employed on the Continent for brewing lager beers. It is commenced at a lower temperature than the infusion mash and raised to the "saccharification" temperature by boiling one or more portions of the mash and returning them to the main mash. The refractory starch is gelatinised by boiling and converted when returned to the main mash, in which diastase is still active. The starch of unmalted cereals must be prepared in some way for conversion, either in the course of manufacture or by boiling. Flaked materials and certain prepared grists are ready for mixing with the malt and can be used in suitable proportions with malt in an infusion or decoction mash. Rice or maize grits must be boiled to gelatinise the starch before it can be converted by the enzymes of malt. This operation forms an integral part of the brewhouse process, when these materials are used in the double mash system. Since a portion of the malt is mixed with the grits to facilitate gelatinisation, and the boiling mixture is added to the main mash, thereby raising its temperature to that required for conversion, the process comprises some of the features of the decoction system, though it is here for convenience described as a distinct system, the double mash, developed in America to deal with highly diastatic and nitrogenous malts with which a fairly high proportion of grits could be used with advantage.

A greater proportion of the protein digestion is effected during malting by the British system than in that used for making lager malts. Comparatively little further degradation is required in the mash tun and the temperatures of 145°–155° used in the infusion system are appropriate. Greater reliance is placed on the mash for protein degradation in the less fully modified lager malts and, since this occurs most actively at temperatures around 122° F., the lower temperature at which a decoction mash must be started to permit of the addition of boiling mash without exceeding the saccharification temperature, makes it more suitable for malts of this type. The ordinary brewing malts of America, the Manchuria type, contain a very high percentage of nitrogen and have high enzymic activity. They are consequently well adapted to use with grits which yield no nitrogen to the wort.

The composition of the worts obtained by the infusion and decoction systems differs to some extent. There is a longer continuance of enzyme action in the infusion mash and no deliberate attempt to arrest it by high temperatures previous to sparging. As a result the wort generally contains a higher proportion of maltose than that from a decoction mash, in which a greater quantity of the higher starch and protein digestion products usually exist. More reliance is placed on them for producing palate fulness in lager beer than in ales, to which flavour is given by a higher hop rate. The wort of an infusion mash is typically more attenuative, the higher alcohol content of the ale adding to the sense of palate fulness. These properties distinguish typical ales and lager beers, but it is, of course, possible to vary the fermentability and composition of either type of wort by suitable adjustment of temperature and other mashing conditions. The possibility of varying the wort composition by adjusting the proportions of the boiling grits mash and the main malt mash or the original temperature of the latter is an important feature of the double mash system.

THE INFUSION MASH SYSTEM

(402) Mashing Plant.

The plant used for an infusion mash consists essentially of a mash tun only, but an external " mashing machine " is almost invariably used to ensure intimate mixture of the grist and liquor. The mash tun is usually, though by no means always, fitted with internal raking machinery and generally delivers into an underback and not directly to the copper. It is simpler than that used in the decoction system and does not so well lend itself to the elaborate decorative schemes adopted in many lager brewhouses. Nevertheless, a large mash room may be very impressive, as shown in Fig. 92.

The mash tun is generally made of cast iron, lagged and cased in wood, though copper is not infrequently used and wood persists in some breweries. Covers of copper or iron and, most recently, stainless steel are usually fitted. These are designed to be lifted up in one piece or in sections and are frequently fitted with inspection windows and internal lamps to facilitate inspection. Some brewers prefer stout, wooden covers of teak, as they provide better heat insulation than metal and minimise the drip of condensed water. The internal mashing machinery usually consists of rakes, revolving on a horizontal axis which is caused to travel slowly round the tun by a central drive, making 1 to 1½ revolution a minute. This simple arrangement is not very efficient for mixing the mash, which should be effected by the external mashing machine and would be difficult to assure otherwise, with the very thick mash usually adopted. The rakes are, however, useful for loosening the goods in the event of a " set mash " and helpful when an underlet is used. They are generally sent round a few times immediately after mashing. Many brewers, however, prefer to dispense with them entirely as they increase the tendency to channelling in the goods and thereby cause unsatisfactory extraction during sparging. Occasionally, grains-removing arms are also fitted in the tun, but the grains are still most frequently shovelled through a slide in the bottom of the tun.

Most of the objections to rakes are overcome in new designs, first used in decoction lauter tuns. The revolving rakes are replaced by knives, which may be turned at right angles to the arms, to cut through the goods as they move round the tun, or parallel with them to turn the grains out. The machinery is operated from below and can be revolved at varying speeds. It can also be lifted vertically by hydraulic power, carrying the sparge arms with it. The latter are delicately pivoted and caused to rotate by the weight of water delivered from a central cup-shaped vessel. In some designs, the sparge liquor is delivered from a pipe along the top of the machine. At the commencement of sparging, the knives are level with the surface of the goods, but can be made to descend very slowly, removing at each turn about ½ inch or an inch of the grains and throwing them out through conical outlets. In this way the grains are removed *pari passu* with the sparging until, finally, it is only necessary to flush out the tun with liquor.

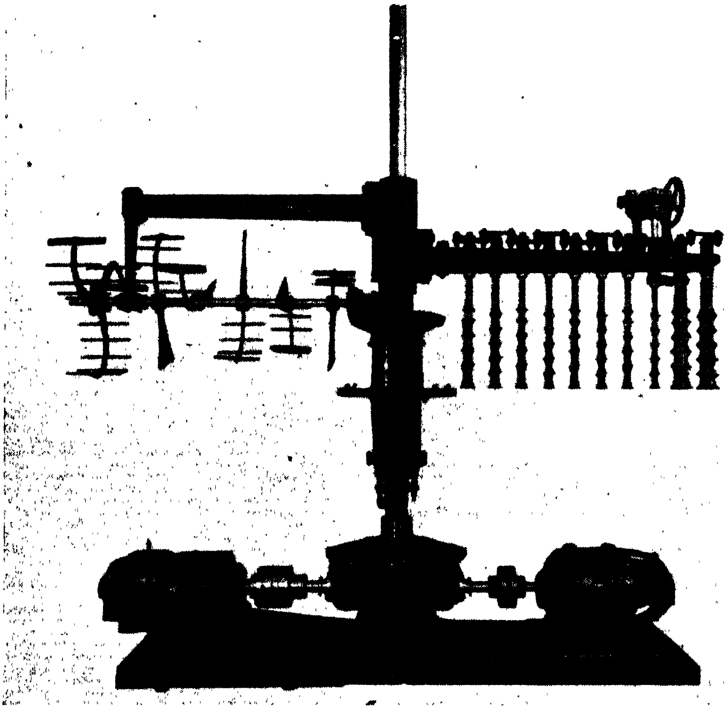

Fig. 91

MASHING MACHINE (WITH KNIVES AND RAKES)

An American machine with both knives and rakes, suitable for use with a depth of goods not more than 28 inches, is shown in Fig. 91. The sparger is suspended on rods from the top of the tun and is caused to revolve on stainless steel balls and race by a liquor pressure of 4 lb. per square inch. The machine is raised to its highest position when the sparge is started, and gradually lowered to reach the lowest point when sparging finishes. The entire machine revolves once in three minutes, and the bevel gears which operate the propeller mashers revolve this shaft at about 1 r.p.m., at which speed the machine does not affect the filter bed. The rakes work at a level about 3 inches above the bottom of the knives. A regulating device is provided for turning the knives to discharge the grains. The larger motor is used during mashing and for removing the grains. The smaller motor is used during sparging.

Important points in mash tun design, to secure a rapid separation of the wort without loss of extract, are the construction of the false bottom, correct distribution of spend pipes and avoidance of suction at the taps, correct size in relation with the quantity of malt mashed and even distribution of sparge liquor in a gentle spray. The false bottom is constructed of accurately fitted gun-metal plates rebated to interlock and supported on short feet. The slots in the upper surface are sufficiently narrow to prevent the passage of grains, widening out below to prevent choking. They must be kept clean and free from scale by soaking in 10% caustic soda solution when necessary. Sparge arms are carried on special bearings, centred over the tun, and the arms are caused to revolve by the centrifugal force set up by the head of liquor supplying them and delivered in a fine spray through perforations along their length. The perforations are more widely spaced towards the centre of the tun, so that the surface of the goods shall be uniformly sprinkled. This may be tested by placing a line of buckets or a wedge-shaped wooden trough with transverse partitions on the bottom of the tun, extending from its centre to the periphery, and noting that an equal depth of water is discharged into each bucket or division of the trough when the sparge is revolved a few times. Care must be taken that the perforations of the sparge arms do not become enlarged by wear or cleaning. A heavy spray forces down the goods and impedes the running-off of wort.

Steel's mashing machines are generally employed for external mixing of the grist and liquor. They are connected directly to the grist case, a screw-manipulated slide closing the chute down which the grist falls and serving as a flow regulator. The liquor main enters the side of the machine and is fitted with a thermometer, as shown in Fig. 93. Frequently, as in this case, one machine is made to serve two tuns by means of a movable spout. Intimate mixture of the grist and liquor is secured by internally revolving rods, while the mixed grist and liquor are carried forward by a short screw.

The wort is usually run off from the tun through a number of holes, evenly spaced in the bottom of the tun and connected by pipes to taps delivering into an open copper trough, from which

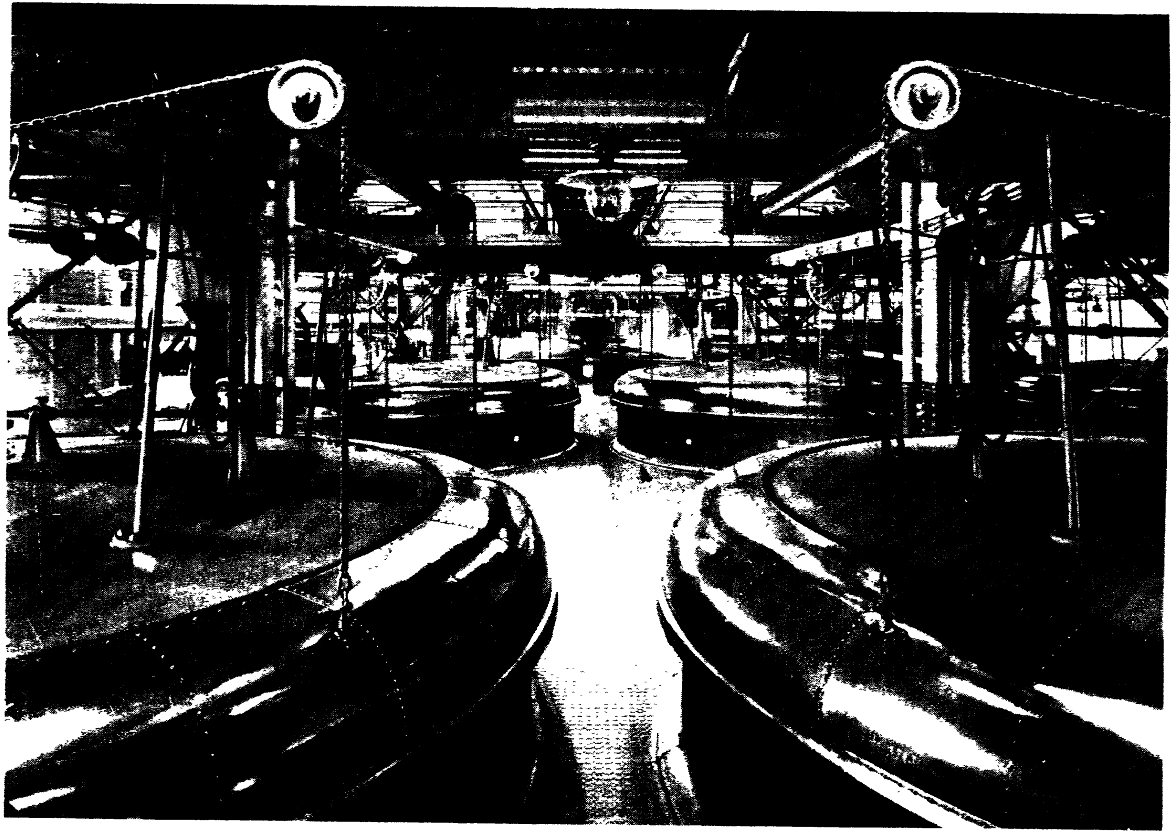

Fig. 92

MASH ROOM (ARTHUR GUINNESS, SON AND CO., LTD., DUBLIN)

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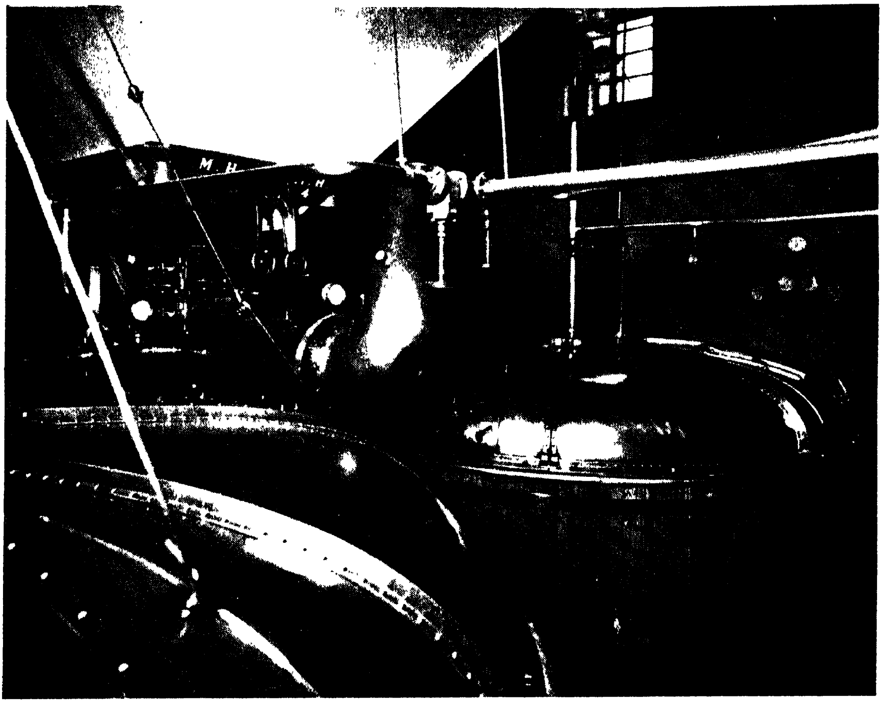

Fig. 93
STEEL'S MASHING MACHINE (WILLIAM, YOUNGER AND CO., LTD., EDINBURGH)

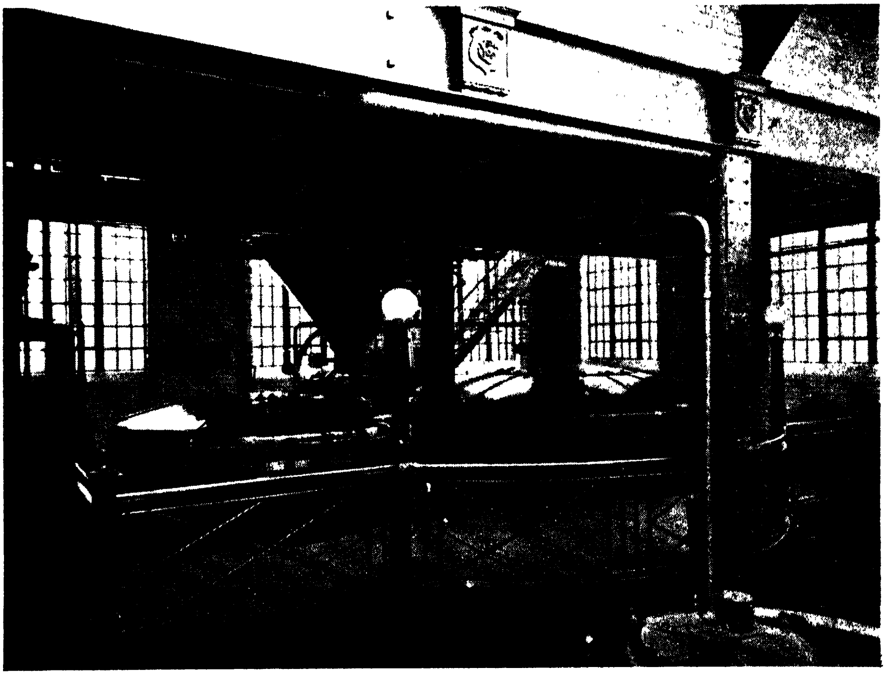

Fig. 94
MASH TUN STAGE (CATTERALL AND SWARBRICKS BREWERY, LTD., BLACKPOOL)

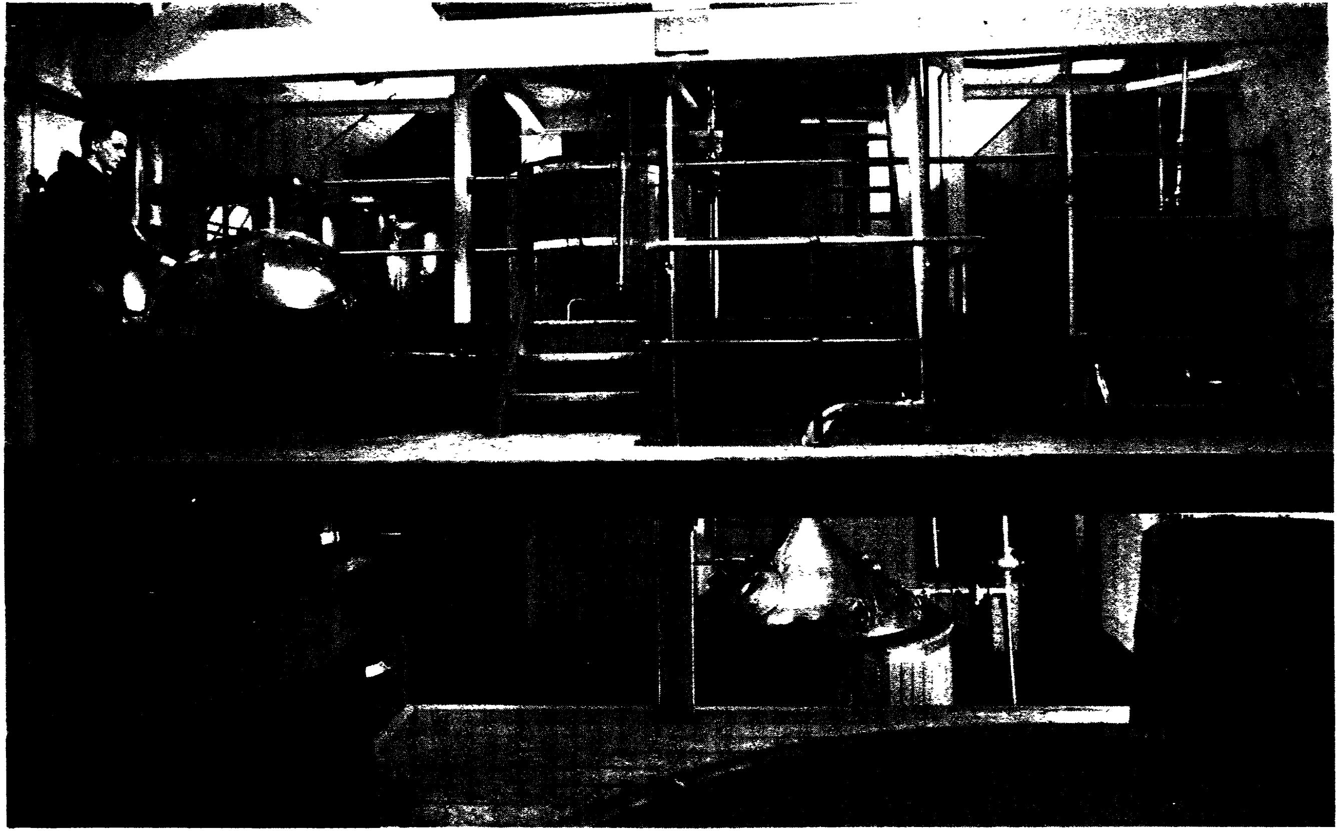

FIG. 95

EXPERIMENTAL BREWING PLANT (J. W. GREEN, LTD., LUTON)

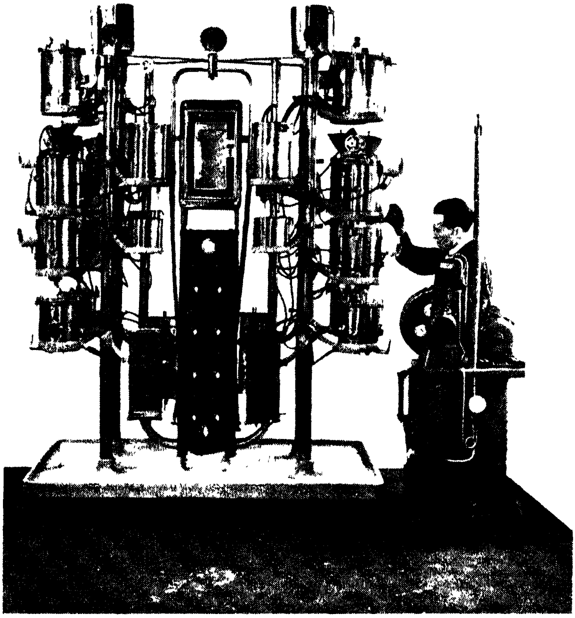

FIG. 96

EXPERIMENTAL BREWING PLANT

(INSTITUTE OF BREWING RESEARCH LABORATORIES)

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the wort can be circulated through the bed of grains, if necessary, until it runs brilliant and is fit to pass to the underback or copper. In most American breweries a closed, cylindrical wort receiver or " grant " is used instead of the open trough. The wort mains are fitted in such a way that hot liquor or " underlet " can be passed upward, through the false bottom into the mash to increase its temperature, raise the goods or clear the slots of the false bottom. The taps are generally adjusted individually, to equalise the running-off of worts from different parts of the tun, but manometric devices for preventing undue suction of the wort, with consequent settling of the goods and choking of false bottom plates, are useful. These may be very simple, consisting of a syphon tube rising to the height of the goods, with a valve on the top and another on a short straight length of the main at the base of the syphon for drawing off the last worts. In some cases these syphons are arranged to pivot, so that the top can be set at any desired level. A complete mashing plant is shown in Fig 94.

Technical Terms.—The following technical terms are used in English breweries :—

Liquor heat or striking heat. The temperature of the liquor at mashing. The former term is frequently used for the temperature of the liquor in the tank, the latter is the temperature of the liquor, taken at the mashing machine.

Initial heat. The temperature of the mash, taken in the tun 5 or 10 minutes after completion of mashing.

Sparge heat. The temperature of the sparge liquor.

Tap heat. The temperature of the wort running off at the taps, usually taken 10 or 15 minutes after " setting taps," that is after the taps are opened. Reference may be made to the first, second or third taps if the wort is run off intermittently.

Mash rate. The relative quantities of liquor and malt mashed, usually expressed in terms of barrels per quarter.

Underlet. Liquor run into the mash through the false bottom of the tun, usually to raise the temperature of the mash.

Goods. The mash in the tun.

Dead or set mash. A mash in which the goods have settled down so heavily and compactly as seriously to retard running-off of worts.

Flooded mash. A similarly defective mash, covered entirely or in places with liquor.

(403) Mashing Procedure.

The mash tun is heated approximately to the mashing temperature by steam or hot liquor. A thermometer is occasionally fitted to the tun to check this, otherwise the hand is used. The liquor temperature is checked at the tank, the gauge read and sufficient mashing liquor run into the tun to cover the false bottom plates. The temperature at the tank is higher than the striking heat by the amount known to be lost between the tank and mashing machine in the particular plant. The striking heat may be 4° or 5° F. higher than the initial heat. Cold and hot liquor supplies are only occasionally available for adjusting the striking heat at the tun. Both hot and cold liquor should have been treated in the same way, if they are used.

The brewer, being assured that the outlet valves of the tun are closed, opens the liquor valve of the mashing machine and then the grist slide, the latter slowly at first and until the flow of malt and liquor is regular, when the thickness of the mixture is regulated by sight and sound of the mash entering the tun. This is constantly watched to ensure an even mash and the striking heat observed. After the mash is all in the tun, a signal being received that the grist case is empty, the grist slide is closed, the mashing machine flushed with liquor, the valve closed, the liquor gauge read, and the mash rate checked. If the tun is fitted with rakes, these are revolved for a short time and the initial heat taken with a registering thermometer at several points in the tun. If there is no underlet, the mash is allowed to stand for 2 or 2½ hours before the taps are set. If underlets are used, the times of the various stages in the mash may be somewhat as follows :—

(1) Mashing about 15 or 20 minutes.
(2) Stand, 30 minutes.
(3) Underlet, sometimes with sparge running.
(4) Stand 1½ to 2 hours.
(5) Set taps and sparge.
Total time, 6 to 6½ hours.

Temperatures are controlled by fixed thermometers on the liquor tank, inlet main to the mashing machine and, sometimes, at the outlet of the latter. Dial thermometers are more easily read than the ordinary mercury type. Like all others, they should be periodically checked against a standard instrument and allowance made for any errors detected. They often read too high after having been in use for some time. A steel-encased bulb is most convenient on the registering initial-heat thermometer. Recording thermometers are of little value in the usual infusion mash tun, as the rakes are not an efficient means of securing an even mixture and temperatures are liable to vary quite considerably at different parts of the tun when the initial heat is taken. It is therefore usual to average readings obtained at several places in the mash. A recording thermometer should, however, be fitted whenever thorough mixture can be assured, as the graph provides the best record available.

(404) Mashing Temperatures and Rates.

The initial temperature is the most significant when no change is made during mashing. A variation of a degree or two has a quite definite influence on the composition and properties of worts from a given malt. The diastatic activities of dark or mild ale malts are lower than those of pale malts and consequently lower mashing temperatures are employed with the former to obtain equally fermentable worts. Grists containing a proportion of black or crystal malts would require a still lower mashing temperature, unless, as is usual for stouts, they comprise a fairly liberal proportion of pale malt. The extent and rapidity of conversion is, however, influenced to a greater extent by the readiness with which the malt is converted than by its diastatic activity, when this is within the rather narrow limits usually met with in practice.

It is frequently assumed that saccharification is completed in about 20 or 30 minutes from the time at which the mash is all in the tun. Iodine solution should by then give no colour reaction with a sample of the mash, but hydrolysis still proceeds more slowly (Section 442) and maltose production is materially influenced by the time at which an underlet is given and the temperature to which it raises the mash. It is increased by prolonging the stand to 45 minutes or reduced by underletting after 15 minutes, instead of 30. The lower initial heat with subsequent increase to a temperature higher than would be employed without an underlet also facilitates the conversion of less well modified malts, and this method of working is preferable with such malts to a higher initial, such as can be used with very friable, more fully modified malts. The same apparent maltose percentage can be obtained with both methods of mashing, though the initial would be two or three degrees higher when no underlet is used.

Varying degrees of fermentability are required in different kinds of top fermentation beer and are secured by suitable variations in mashing temperatures, decided in accordance with the grist used. Low mashing heats are used for highly attenuating worts, but too low initials tend to give worts in which the fermentations will drift instead of finishing in a normal manner. Wort containing a greater proportion of dextrins is required for stock ales. This demands a higher mashing temperature, even with the dark malts used for strong ales. A protein digestion at 120°–130° F. is rarely adopted in British practice. The malts are usually well modified and contain a sufficient proportion of ready-formed soluble nitrogenous substances, but a low temperature mash and stand can be given if required with under-modified malts. Temperatures above this but below about 150°, if held for the entire mashing period, are liable to yield worts with an unduly high percentage of maltose and ill-adapted to top fermentation conditions. It should be noted, however, that mashing temperatures of 145°–155° F. do not preclude proteolytic action in the mash tun and that a certain amount of protein digestion does occur.

Grists and mashing conditions are so varied that it is impossible to lay down definite rules for temperatures and procedure. In any one brewery, the temperatures are constantly subject to check against the wort analyses and are varied as circumstances dictate with changing grist, to keep the wort composition as constant as can be judged by the available methods of analysis and, more particularly, in accordance with the behaviour of the wort in the fermenting vessel. The figures given in Table 159 must therefore be regarded as no more than typical examples of mashing temperatures and rates for different types of beer. Underlets may or may not be used for any of these, while some brewers might use two underlets at increasing temperatures, with a stand and, possibly, a short sparge between them. Temperatures higher by two or three degrees are commonly adopted at the commencement of sparging to compensate for loss of temperature in the mash, and, in the case of stouts, very considerably higher temperatures are sometimes allowed at the later stages. The underlet temperatures are taken in the mash. That of the underlet liquor may be about 180° F. The sparge temperature is that of the liquor.

TABLE 159.—INFUSION MASH DETAILS

Type of Beer	Mash		Underlet		Sparge	
	Rate	Initial temp. F.	Rate	Temperature of Mash	Rate	Temperature of Liquor
Pale ale ..	2½	152°	nil	—	4	168°
Stock beers ..	2	153°	1	156°	3½	168°
Light bitter ..	2¼	148°	1	154°	3½	168°
Mild ale ..	2¼	147°	1	153°	3½	168°
Stout	2¼	146°	1¼	154°	3½	170°

(405) Infusion Mash with Raw or Prepared Grain.

It is common practice to use flaked maize or rice in an infusion mash, in quantities from 5 to 15% of the total grist. The starch of these materials is very readily converted in the mash tun and all that is necessary is to blend them thoroughly with the ground malt as it enters the grist case. Simple machinery is available for this purpose. In other breweries, maize or rice grits are used in about the same proportions. These must, however, be gelatinised by boiling before they are mixed with the malt mash. One method adopted is that described in the next Section on the Double mash process. In other breweries, the gelatinised and liquefied grits mash is reduced to mashing temperature and used in place of liquor with the Steel's mashing machine.

SPARGING

(406) Running off the Wort.

The final stage in the mashing process, in which the wort is run off and absorbed wort washed from the grains as completely as possible, without extracting substances from the latter which might deleteriously affect the quality, brilliance, stability or flavour of the beer, is carried out in a similar manner in all systems. The mash tun, almost invariably, serves as a clarification or filtration vessel in the infusion system, the liquor required for washing out absorbed wort being evenly sprinkled over the goods by means of revolving sparge arms. The goods, to which the malt husks give porosity, are prevented from being washed into the spend pipes by the perforated false bottom of the tun and act as filtering medium to retain the sludge of finely divided unconverted material and coagulated protein matter produced during mashing. It is usual in the decoction system to mash in one vessel and afterwards pump into another for settling and filtration, and in some breweries a mash filter is used in place of a filtering tun.

Despite the high temperature to which the mash has previously been raised, a certain amount of diastase may still remain, particularly in an infusion or double mash, and be capable of acting on any small quantity of starch that may exist in hard-ended or steely corns. Since the liquefying function of diastase survives temperatures which completely destroy the saccharifying activity (Section 439), there is a risk of liquefying starch with failure to saccharify it, if the sparge liquor is at too high a temperature, an occurrence which is detected by the production of a blue or brown colour on addition of iodine solution to the last runnings. The action of diastase is inhibited by high concentrations of maltose, but the dilution that occurs during the later stages of running off restores conditions under which undestroyed α-amylase can resume its activity. It is common practice to sparge with liquor at a temperature of 168°–170° F., though many brewers start at about 167° and gradually rise to higher temperatures, while it is desirable in some cases to start at a somewhat higher temperature to raise that of the cooled surface of the mash before dropping to 167° or 168°.

The efficiency of the sparging process depends on the evenness and gentleness with which the spray of liquor is distributed by the sparge arms over the surface of the goods, the regularity of the filter bed of grains, absence of channels down which the liquor can run and its depth, which should not exceed about 3 ft. 6 ins. Two taps are first opened to clear the bottom of the tun, the first runnings of cloudy wort being pumped back if necessary and possible. The taps are all opened to about ¼ of their full extent when the wort runs bright. The flow is gradually increased and the taps adjusted so that all run evenly. The gravity of the wort should be the same at all the taps, indicating even running over the entire surface of the false bottom. Individual taps are opened or closed slightly as may be necessary to adjust this. If the tun is not fitted with some type of grains-removing device, the level of the wort is held at the surface of the grains by sparging at the same rate as the wort runs off. Automatic manometric apparatus is available to keep a steady level and prevent undue suction, which prevents efficient working. Sparging is continued until the last runnings have a specific gravity of 1002–1003, providing they are free from starch. The speed at which the worts run off is dependent on many factors, among which are the modification and grinding of the malt, the compactness of the grains and the temperature. It is desirable to prevent fall of temperature by good insulation of the tun.

A slow-running wort may be due to (*a*) too fine grind, (*b*) excessive raking, which causes the sludge to separate and clog the slots of the false bottom, (*c*) clogging of slots which have become enlarged through long use. A " dead mash " results from settling of the goods into a tightly packed mass It becomes obvious through slow running and collection of liquor on the surface of the mash, or flooding. It may be caused by (*a*) too long stand, (*b*) running worts too rapidly, or (*c*) sparging too fast, which sucks or beats down the goods, (*d*) steely malt, (*e*) too deep a tun. If there is a tendency to slackness in running, the sparge may be stopped, the rakes revolved once or twice and the mash rested a short time before resumption of sparging. In other cases it may be necessary to give a sudden, sharp underlet to clear the false bottom slots. Sparging may be continuous during running off but, if an unusually strong wort is required for high gravity beers, the first worts are run off until the flow from the taps slackens before the sparge is started. Some brewers then make a second mash with underlet and give a short rest before resetting the taps.

It is desirable to limit the sparge liquor, so that the total quantity used for the mash and sparge together shall not exceed 6½ or 7 barrels per quarter of malt. The gradually increasing temperature in the grains results in the extraction of colloidal carbohydrate and protein matter, which gives an opalescence to the wort, accompanied by an increase in its p_{H} value, if carbonate liquor is employed. The unconverted colloidal matter carried forward is of no advantage and may prove troublesome in respect of beer brilliance. The slight loss of extract involved in rejecting runnings with a lower specific gravity than 1003 is usually more than counterbalanced by improved quality of wort and beer. Some brewers hold over runnings that cannot be used without reducing the gravity of the worts too much and mix them with the mashing liquor of the following brew. This practice saves a certain amount of extract, but bacterial growth may be troublesome in warm weather, unless steps are taken to sterilise the mixed runnings and liquor immediately.

The wort is run from the mash tun to an underback, which, in some breweries, is little more than sufficient to serve the pumps to the coppers. It is more frequently a large cast iron tank, fitted with steam coils to keep the temperature of the worts above 180° until they are pumped up and so prevent further enzymic action.

CONTROL OF MASHING

(407) Analysis of Wort.

A complete analysis of wort is not possible with the methods yet available and, in any case, methods of analytical control in manufacturing processes must be reduced to their simplest terms, consistent with reliability within the requisite limits of accuracy. Analysis of the product must be rapid and is frequently restricted to empirical methods. Mashing control is, consequently, limited in most breweries to observations in the mash room, supplemented by determinations of the optical rotatory or reducing power of the wort and, less frequently, its reaction and nitrogen content. Though the information on wort composition gained by these methods is vague, the data are very useful when consistently obtained and compared. Isolated determinations are of much less value. Observations can be carried out as part of mash room routine or in the laboratory to obtain information on the following points :

(1) Temperature in the mash tun and of the wort at the taps.
(2) Brilliance and colour of the worts.
(3) Specific gravity or extract at various stages of running off.
(4) Iodine test for starch and dextrin with the mash and wort, particularly last runnings.
(5) Apparent maltose content of the wort by reduction of Fehling's solution (Vol. I, Section 247).
(6) Optical activity or $[\alpha]_D$ of the wort solids (Vol. I, Sections 244–246).
(7) p_H value of the wort at different stages, usually determined colorimetrically though this method is not very reliable on account of protein errors (Vol. I, Sections 100–102).
(8) Titration acidity and buffering power of the wort.
(9) Residual extract in the grains.

The following are more usefully determined in the copper wort :

(10) Permanently soluble nitrogen.
(11) Coagulable nitrogen.
(12) Amino-nitrogen.
(13) Limiting attenuation:

(408) Routine Observations.

The striking and initial heats of the mash are always recorded, the latter being the most significant guide to wort composition. The mash temperature is again taken after an underlet. Great weight is also usually attached to the tap heats, as they are not subject to the uncertainty attending the ascertainment of the initial heat or second mash heat. Experience soon establishes the relation between the tap and initial heats or between the tap heat and the higher temperature reached in the tun after underletting, in any particular plant. Constant temperatures from mash to mash with the same grist and mashing rate should mean regularity in wort composition, but some alteration in mashing temperatures is usually required to maintain this when any change is made in the malt. These are so liable to occur between different strippings of the same malt or in large bulks that constant comparisons between mashing temperatures and wort analyses should be made. Wort should be brilliant as run off from the mash tun and means are usually available for recirculation of the first few barrels over the goods, if this appears necessary. This is the first of a series of observations on wort made at the different stages of brewing. Some veiling generally occurs with the weak runnings, but persistent haze in the stronger worts suggests inadequate conversion of steely malts, and considerable veiling during sparging indicates excessive liquor temperature or undue increase in the p_H value of the wort, due to use of untreated, alkaline liquor and extraction of undesirable constituents of the grains. Such indications may be the precursor of defective break in the copper or during cooling, and of lack of brilliance or stability in the beer. Channelling of the goods, with sucking down of sludge, will also be detected by the appearance of " bits " in the samples taken during running off. Should this occur, the taps are shut off for a time and the rakes put round if required. It may be necessary to re-circulate for a short time when the taps are again set.

It is rarely necessary to determine the colour of worts with any greater accuracy than is afforded by observation of the sample glass, which is sufficient to make sure that no mistake has been made in the malt or otherwise. The specific gravity must, however, be taken from time to time during running off to make sure that the sparging is effective and as a guide to making up the copper or coppers. Samples should be taken from all the taps to find whether the wort is running regularly. Low gravities at individual taps suggest channelling. The time for closing the taps is also determined by the specific gravity reaching the point allowed for last runnings, not usually lower than about 1003. The last runnings are always tested for dextrin and starch by adding a few drops of a strong solution of iodine to a sample in a glass or by a drop test on a white tile. No brown or, more particularly, blue colouration should be produced.

(409) Limitation of Extract.

It is not usually considered advisable to push extraction to the limit obtainable by very prolonged sparging, since this involves extraction of incompletely converted carbohydrate and protein matter. These objections do not apply to the greater extracts obtained by modern improvements in milling plant and mash tun operation, and the tendency is to get as much extract as possible by these means. Little or no deterioration in beer quality results, provided the sparge liquor is suitably treated to prevent undue increase of p_H value of the later runnings. The extract is, however, limited by the coarseness of grind necessary to permit of rapid filtration and prevent reduction in the buoyancy of the goods. The extract remaining in the grains usually amounts to between 1·0 and 1·5 lb. per quarter of malt used.

(410) Extract Loss in Grains.

Loss of extract may be caused by incomplete conversion, due to faulty malts or mashing, and by defective sparging. It should not exceed 2 lb. per quarter, but may be as much as 5 or 6 lb. per quarter of malt in bad cases. The total loss may be determined with sufficient accuracy on the assumption that 50 grams of malt gives 15 grams of dried grains or 60 grams of wet grains, as taken from the tun. These weights of dried or wet grains are mashed with 5 ml. of cold water malt extract and 250 ml. of distilled water, raising slowly to boiling point and boiling until gelatinised, as in the analysis of raw grain. The mash is then cooled to 150° F. and mashed for 1 hour at 150° F. with a further 45 ml. of the cold water malt extract, cooled to 60° F., made up to 515 ml. and filtered. From the specific gravity of the filtrate, that of a control mash with the malt extract and water alone is deducted. The difference, multiplied by 3·36, gives the total loss of extract.

The sparging loss is determined by thoroughly mixing 15 or 60 grams of dried or wet grains, respectively, with distilled water in a 515 ml. flask and making up to volume at 60° F. The mixture is filtered and the specific gravity of the filtrate determined. The sparging loss is then calculated from the expression (sp. gr. — 1000) × 3·36 = lb. extract lost per quarter of malt. The loss due to incomplete conversion is then found by deducting the sparging loss from the total loss.

ANALYTICAL METHODS

(411) Optical Activity of Worts.

The optical rotatory power of wort (Vol. I, Sections 244–246), is determined most conveniently by means of a saccharimeter, using white light, and calculated as $[\alpha]_{D3\cdot86}$. The sample, taken 15 minutes after setting taps, is immediately boiled to arrest enzymic action. It is then diluted until its specific gravity is

1038–1040, fined and filtered for examination in a 1-dm. tube, the specific gravity being determined with a specific gravity bottle. A stout wort can be bleached by addition of a few ml. of a solution of bleaching powder.

It is practically impossible to correlate the optical activity of wort with those of its known constituents, but it is always much below that of a mixture of maltose and dextrin. Some part of the difference is due to glucose, to optically inactive salts and to proteins, which may have a negative rotation. It is also impossible to give an optimum $[\alpha]_D$, applicable to one type of beer in every brewery, or to state, for example, that an $[\alpha]_D$ of 116° or some other value should be best for pale ales in a particular brewery, because it happens to be most suitable in another. Neither is it possible to decide from a single determination in a strange brewery whether the indications given are favourable or not, unless the result is unusually high or low. The value of the determination depends on experience of the conditions prevailing, which can only be gained by co-ordination of a series of results with the subsequent behaviour of the wort and beer.

An $[\alpha]_D$ of 114°–118° is typical of all-malt infusion worts. A lower figure, such as 110°–112°, suggests a greater proportion of maltose and corresponds with a more fermentable wort, while figures above 120° indicate more highly dextrinous, less fermentable worts, suitable for stock beers. A difference of about 2°–4° is commonly found between the $[\alpha]_D$ of pale and mild ale worts, the pale worts giving the higher figure. If maize or rice is used, the $[\alpha]_D$ will be about 1° higher for each 4 or 5% in the grist, so that an $[\alpha]_D$ of 120°–122° may then represent a readily fermentable wort. A variation from the value normal to a particular type of wort in one brewery may indicate a change in the composition of the grist or in mashing conditions. Changes in the composition of the malt, drawn from the same bin, can be detected in this way and allowed for in the next brew by appropriate changes in mashing temperature. If the variations cannot be traced to the malt, they have probably been caused by changes in mashing temperature or in the mashing rate that may have escaped notice in the brewhouse.

A fall in $[\alpha]_D$ may indicate (*a*) slack malt, (*b*) higher diastatic activity, (*c*) more than usually full modification, with a high percentage of ready formed sugars, (*d*) lower mashing temperatures or (*e*) low mash rate. It can be adjusted in the next brew, to correct the increased fermentability that will probably follow, by raising the mashing temperature with the same malt, by changing the malt or by attention to any fault in the brewhouse that may have led to a reduced mashing heat. An increase of 1° or even ½° F. in the mashing temperature may be sufficient to produce the required result.

A rise in rotatory power, due to the production of more dextrinous and less fermentable wort, may have resulted from (*a*) a malt of lower diastatic activity, (*b*) insufficiently grown or under-modified malt, (*c*) higher mashing temperatures, or (*d*) extra liquor in the mash, above that usual in the standard mashing rate, such as may have resulted if the flow of grist to the mashing machine had been impeded. A reduction in mashing temperature or greater regularity in mashing is indicated for the next brew with the same malt.

(412) Reducing Power of Worts.

The cupric reducing power of wort is determined, after suitable dilution, by one of the various methods of estimating reducing sugars, usually by means of Fehling's solution. The gravimetric and volumetric methods described in Section 247 are suitable for this purpose. The cupric reducing power must be expressed in terms of some single reducing sugar, for reasons given in Vol. I, Section 247, and maltose is the obvious choice for wort analysis. The results are usually given as a percentage of the wort solids, but they do not represent the true maltose content of the wort, since other starch conversion products and any glucose and pentose that may be present also reduce Fehling's solution. They are consequently expressed as "apparent maltose." The dry solids of the wort are computed from its specific gravity at 60° F. by means of the conventional sugar divisor 3·86 (Section 241) or, more accurately, by dividing the excess gravity over 1000 by 3·95 or 4·0. This gives grams of dry wort solids in 100 ml. Alternatively the wort solids, as grams per 100 grams of wort, may be derived from the specific gravity at 20° C. and the Plato tables, and the weight-volume percentage calculated from this, if desired.

Pale ale and pale lager worts usually contain from 65–72% of "apparent maltose" on wort solids, mild ale worts about 63–68% and those of dark lagers and stouts 55–65%. The percentage may be taken as an approximate indication of the fermentability of the wort, high figures corresponding with highly attenuative worts and low figures with less fermentable. Similar deductions can be drawn from variations from the normal "apparent maltose" of worts as from its $[\alpha]_D$, an increase corresponding with a fall in $[\alpha]_D$ and a decrease with rise in the latter. It is possible to make some allowance for the fermentability of the wort by increasing or decreasing the yeast pitching rate or pitching temperature, as indicated by the results of eith of these determinations.

The remainder of the wort solids, after deduction of the "apparent maltose," is frequently referred to as "non-sugars" and assumed to consist of proteins, ash, non-reducing carbohydrates or dextrins, etc. A "sugar" : "non-sugar" ratio is then given to represent the composition of the wort in an approximate manner. Pale worts with 65 or 72% of apparent maltose on wort solids would thus be represented as having a sugar : non-sugar ratio of 1 : 0·54 or 1 : 0·4, respectively, and less fermentable worts with 60–65% of apparent maltose would have a sugar : non-sugar ratio of 1 : 0·66 to 1 : 0·54. It will be recognised that this is an entirely empirical convention, bearing little relation with the actual wort composition. A considerable proportion of the reducing power, assigned to maltose, may be due to dextrins (see Section 414) and a number of other wort constituents tend to upset the assumed parallelism between reducing power and fermentability. Reducing dextrins or, as they are still frequently styled, "malto-dextrins" are not fermentable by brewery yeast, and cane sugar, which has no reducing power and may account for some 7% of the wort solids, is fermentable.

(413) Limiting Attenuation.

Since the $[\alpha]_D$ of wort and its apparent maltose content do not give a true indication of its fermentability, it might be thought better to measure this directly by small experiments with yeast. Though this is sometimes done, its practical value for mashing control is limited by the time required for the fermentation and it is generally more usefully determined with the collected wort. An obvious difficulty arises if the wort is boiled in more than one length, in which case a sample corresponding as closely as possible with the mixed worts in the fermenting vessel should be obtained.

The term "limiting attenuation" applies to the attenuation secured under the most favourable laboratory conditions. It should strictly express the percentage of the original solid matter fermented, the "true limiting attenuation." It is, however, generally used to express the percentage of the original gravity of the wort lost during fermentation, the "apparent limiting attenuation." Sometimes it is loosely employed for the final gravity obtained in the laboratory fermentation, in accord with the meaning of attenuation commonly adopted in breweries.

The determination may be carried out in a simple manner by fermenting 200 ml. of the hopped wort with 0·5 gram of fresh pressed yeast in a conical flask, plugged with cotton wool and kept in a thermostat at 77° F. (25° C.) for 4 or 5 days, with frequent shaking to distribute the yeast and remove CO_2. The original gravity of the wort and its final specific gravity, without removal of alcohol, are determined and the "apparent limiting attenuation" calculated as in the following example, in which the original gravity was 1050 and the final gravity 1012·5:

$$\frac{(1050 - 1012{\cdot}5) \times 100}{50} = 75\%$$

The final attenuation is reached more rapidly if the wort is placed in a rocking apparatus, arranged so that a continuous current of air can be aspirated through it. Certain precautions must be taken if reliable results are to be obtained. Liquid yeast should not be used, while the small addition of pressed yeast may raise the specific gravity by 1001·0, unless the fermented wort is filtered. Slight autolysis of the yeast at 77° F. and acidification may raise the specific gravity of the wort after fermentation. These errors are insignificant if the fermentation time is cut down to 3 days, but may be considerable if it is prolonged. The speed of fermentation can be increased by addition of a little granulated pumice to the wort, which should be sterilised, and carrying out the fermentation at 77° F. in a comparatively large flask, with frequent shaking. The yeast to be tested is pressed and a piece about the size of a pea used with 250 ml. of wort.

(414) Interpretation of Analytical Results.

Attempts to draw conclusions on the composition of such a complicated mixture of unknown constituents as wort (see Chapter XXVII) from the limited data afforded by the $[\alpha]_D$, reducing power and fermentability have so far not proved successful. Such attempts must be empirical and based on assumptions that may or may not be true. For example, it was formerly the custom to assume that the carbohydrates of wort consisted of maltose and dextrin only, ignoring the cane sugar and other carbohydrates which, with nitrogenous substances and salts, are soluble in cold water and may account for 25% of the total extract of the malt. It was further, quite unwarrantably, assumed that all the reducing power was due to maltose and that a definite $[\alpha]_D$ of 202° could be ascribed to "dextrin." On this basis, with an $[\alpha]_D$ of 139° and $R = 100$ for maltose, it is a simple matter to calculate the percentages of maltose and dextrin in the extract and ascribe the remainder to ash, protein, etc. High or low "dextrin rates" would thus indicate less or more fermentable worts, but the results are no more definite than conclusions derived directly from the $[\alpha]_D$

or reducing power of the worts. The figures in Table 160 are calculated on the above assumptions. Though a high $[\alpha]_D$ corresponds roughly with low apparent maltose content and fermentability, the figures serve to indicate the lack of concordance between these figures. The residual apparent maltose represents the reducing power of unfermented dextrins and it is noteworthy how closely this corresponds with the difference between the original apparent maltose and the solids fermented. The latter include small quantities of cane sugar and glucose. The high reducing power of the latter, with the lack of reducing power of cane sugar, account for the correspondence between the loss of " apparent maltose " and solid matter by fermentation.

TABLE 160.—COMPARISON OF REDUCING POWER, $[\alpha]_D$ AND LIMITING ATTENUATION OF WORTS

(Percentages of wort solids)

	1	2	3	4
Maltose, apparent	68·2	66·9	66·2	65·0
Dextrin	11·4	13·9	16·3	18·4
Ash	1·7	1·6	1·5	1·3
Protein (N × 6·25)	4·8	5·2	4·3	4·3
$[\alpha]_D$	114·5	117·6	121·3	123·8
Limit. atten. (real %)	57·9	59·9	58·9	57·4
,, ,, (apparent %) ..	70·7	73·3	71·9	70·6
Residual apparent maltose ..	11·0	8·3	9·9	9·8

Despite such restrictions on their application, and the many unknown factors that may cause variations, determinations of optical activity and reducing power are both useful in practice. There is little to choose between the two analytical methods, when the scope of their indications and their limitations have been learnt by experience. Some brewers prefer to determine the apparent maltose percentage, others rely on the $[\alpha]_D$. Either gives remarkably consistent results when due care is taken to maintain regular grist and mashing conditions. Variations of 1 or 2% in apparent maltose or of 1° or 2° in rotatory power are then significant of some change in the grist or mashing, for which it may be possible to make allowance in fermentation. In many instances the results will be found to be in apparent contradiction with the mashing temperatures. The cause may be difficult to find among the possible unsuspected variations in grist or mashing procedure, but they undoubtedly indicate some change in wort composition.

Some further information on the composition of wort can be obtained by determining the $[\alpha]_D$ and reducing power before and after fermentation, which would remove sucrose, maltose and glucose, and then again after the residue of dextrins, nitrogenous substances, ash, etc., had been subjected to the action of malt amylase. The reducing power after fermentation is assumed to be due to dextrins. It is ascribed to " unfermented maltose " in Table 161. This is increased after degradation of the unfermentable residue by malt amylase by the reducing power due to maltose produced from dextrins, from which the latter may be calculated. The sucrose is determined by inversion. The glucose and maltose are calculated from equations for the reducing power and $[\alpha]_D$ lost by fermentation and the constants for the three sugars. Analyses of this kind are very incomplete and must be more or less unsatisfactory, on account of lack of knowledge of the nature of the dextrins and the assumptions on which the calculations are based, but it is difficult to carry them much further at the present time. The examples by Hagues[2] in Table 161 represent the worts from laboratory mashes of a pale Australian malt and are interesting in showing variations in composition due to the mashing liquor.

TABLE 161.—ANALYSES OF WORTS FROM EXPERIMENTAL MASHES WITH DIFFERENT LIQUORS (HAGUES)

	Carbonate Water, 30 pts. $CaCO_3$ per 100,000		Neutralised Water	
	grm. 100 ml.	% of solids	grm. 100 ml.	% of solids
Maltose	4·10	58·4	4·38	57·6
Glucose	0·18	2·6	0·12	1·6
Sucrose	0·63	9·0	0·57	7·5
Maltose unfermented ..	0·21	3·0	0·16	2·1
Dextrin	0·22	3·1	0·52	6·8
Ash	0·11	1·6	0·12	1·6
Nitrogen × 6·25 ..	0·31	4·4	0·40	5·3
Dry residue	1·26	17·9	1·33	17·5
	7·02	100·0	7·60	100·0

(415) The Nitrogenous Composition of Wort.

Reliance should not be placed solely on determinations of extract and of the carbohydrate composition of wort for mashing control purposes. Its nitrogenous composition ought to be taken into account at the same time. Proteolysis is more readily influenced by variations in mashing conditions than starch conversion and the results have far-reaching effects on the quality of the beer. It is again necessary to make use of simple methods of analysis, by which changes in the proteins and the nature of their breakdown products can only be indicated in the broadest outline. These include determinations of (*a*) the total nitrogen content of the wort, (*b*) the coagulable nitrogen, indicating the quantity of true protein in the form of albumin, (*c*) the permanently soluble nitrogen, and (*d*) the formol nitrogen. The conventional factor 6·25 is generally used to give an indication of the quantity of nitrogen compounds present in any one of these groups. The permanently soluble nitrogen determination is the simplest of these and may very conveniently be expressed as a percentage of the total nitrogen of the malt. This percentage becomes greater as the mashing method, temperature, liquor, p_H value, etc., are more favourable to protein breakdown and allows comparisons of the effects of these factors to be made. It is probably also useful as an indication of the breakdown of hemicelluloses and organic phosphorus compounds under various temperature conditions, since the temperature optima of cytase and phytase are similar to those of the proteolytic enzymes.

(416) Experimental Brewing.

If it were possible to be more definite in regard to wort composition than the present simple methods of analysis permit, and if the influence of the various factors operating in the mash tun could be reduced to mathematical expressions, perfect control of wort composition would depend on the efficiency of the mashing plant, with its temperature and other recording instruments. This is far from being the case at present and many brewers have installed experimental brewing plant, in which they can investigate the influence of variations in materials and processes on the wort and beer. Some of these are capable of dealing with 2 to 10 quarters of materials, but it is possible to obtain guidance on wort composition and behaviour from small laboratory apparatus. The criticism of these experimental plants based on the influence of volume on the quality and character of beer is rather beside the point, since they are not intended to brew beer of identical flavour with that produced in the large brewery. They are used for comparisons of methods and materials and are satisfactory for this, since all conditions and quantities can be accurately controlled. The equipment used in the Institute of Brewing Research Laboratories, shown in Fig. 96, depends essentially on the provision of means for carrying out, simultaneously, a number of miniature mashes with the same or varied grists, under strictly controlled conditions, and for boiling and cooling the wort under equally precise control. The adoption of tall cylinders, vacuum-insulated against external temperature influences, has made it equally useful for fermentation experiments.

The use of plant of this description demands methods of analysis capable of measuring the variations in product resulting from employment of different materials or processes. In advanced experimental work, these would be much more refined than those hitherto described and include methods for studying the behaviour of substances in colloidal dispersion. The simpler methods are, however, adequate for a study of the influence of mashing conditions on wort composition, such as forms the subject of Chapter XXV and is essential to complete comprehension of the principles of mashing.

(417) Summary.

The infusion mash provides the simplest process of wort production. It is particularly adapted for use with well-modified malts, such as are generally used in this country, and has been developed for use with these malts. In its simplest form it consists in the intimate mixture of crushed malt with water at a temperature of about 150° F., approximating to that at which the greatest quantity of conversion products is obtained in the form of extract. This will be shown in a later Chapter to be rather higher than that yielding the maximum quantity of fermentable extract, and considerably higher than the optimum for protein breakdown. Variations in the composition of wort are brought about by mashing at temperatures a few degrees above or below 150°, according as a less or more fermentable wort is required. Further variation in wort composition can be produced by mashing at a lower temperature, with subsequent increase by means of an underlet. This process is particularly suitable for less fully modified malts and, although generally practised, is less necessary than formerly, as present-day malts are, on the whole, more friable than they were when the methods were devised.

A protein digestion can be introduced in infusion mashing, but is usually unnecessary, because protein breakdown during malting is generally adequate. If this were not the case, the malt would be regarded as unsuitable for mashing by infusion. In such circumstances, a decoction mash or some modification of the processes described in the next Chapter would be preferable.

Sparging has been developed, not only to wash all wort from the grains, but also to complete the conversion of hard ends and resistant starch, which exist to some extent even in the most friable malts. If the available appliances are badly constructed or the process is wrongly carried out, there will be a loss of extract through imperfect washing-out of wort, while excessive temperatures extract colloidal constituents from the malt, which prove troublesome in respect of brilliance in wort and beer.

The composition of wort is so complex and imperfectly known that no methods of analysis, suitable for routine control, are entirely satisfactory. Those described in this Chapter, and based on the optical activity of the wort or its cupric reducing power, would be accurate if wort were a simple mixture of dextrin and maltose. They would then give concordant results and quite clearly indicate its fermentability. Wort, however, contains unfermentable carbohydrates which reduce Fehling's solution, and cane sugar which is fermentable but does not reduce it. The optical activities of wort constituents also differ widely, and those of many of them cannot be specifically stated. Hence no true conception of wort composition can be gained by attempting to correlate the results of these two determinations. Nevertheless, determinations of optical activity and reducing power are of considerable practical value and permit of many useful deductions, when their restrictions are properly understood and taken into account.

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1. E. J. Russell and L. R. Bishop, *Journ. Inst. Brew.*, 1933, **39**, 287 (Inst. Brew. Res.).
2. G. Hagues, *Journ. Inst. Brew.*, 1926, **32**, 8 (Inst. Brew. Res.).

CHAPTER XXIV

DECOCTION MASHING SYSTEMS

DECOCTION MASH PLANT

(418) Brewhouse Plant.

Several of the vessels used in a lager brewhouse find no counterpart in the mash room of the ordinary English top fermentation brewery, where the grist is extracted and the wort separated from the grains in a single mash tun. Consequently, the German names with the translations commonly adopted are given to avoid confusion. The word copper is invariably used in England for the vessel in which the wort is boiled with hops, regardless of the material of which it is constructed, and this usage is followed here and the same word is employed for the vessel in which the mash is boiled. These vessels are usually known as kettles in America.

Maischbottich (Mash tun). The vessel in which the mash is made.

Maischpfanne, *Maischkessel*. Mash copper or Mash kettle in which the mash is boiled.

Maischbottichpfanne. Combined mash tun and mash copper.

Läuterbottich. Lauter tun or clarifying tun, identical with English mash tun, but used for settling, drawing off wort and sparging only.

Wurzepfanne, *Hopfenkessel*. Copper, Kettle.

Mash tun.—This differs from the infusion mash tun, in that it is used for mashing only, the subsequent settling and sparging being carried out in another vessel. It is usually connected to the grist case by means of a pipe of sufficient diameter to allow the grist to fall freely to the automatic foremasher or premasher, situated immediately above the tun. The premasher has the external form shown in Figs. 97 and 100. In it, the liquor is intimately mixed with the falling grist by sparging through the centrally situated, perforated end of the liquor main, which is covered by a cone to direct the grist around it. The temperature of the liquor is adjusted by a mixing apparatus, connected to the hot and cold liquor tanks and clearly shown in Fig. 99. A complete mash tun is illustrated in Fig. 97. This tun is fitted with a propeller, driven by directly coupled motor, geared to turn at 6 to 8, 30 to 40, or even up to 100 revolutions a minute, and with mains and pump for transferring the mash into the mash copper or lauter tun. It is also fitted with a jacketed bottom for steam heating, but this is not essential with the complete outfit in which separate vessels are used for mashing and boiling the mash. Like all the other vessels in lager brewhouses, it is surmounted by a copper dome, with a chimney for conveying the steam outside the building.

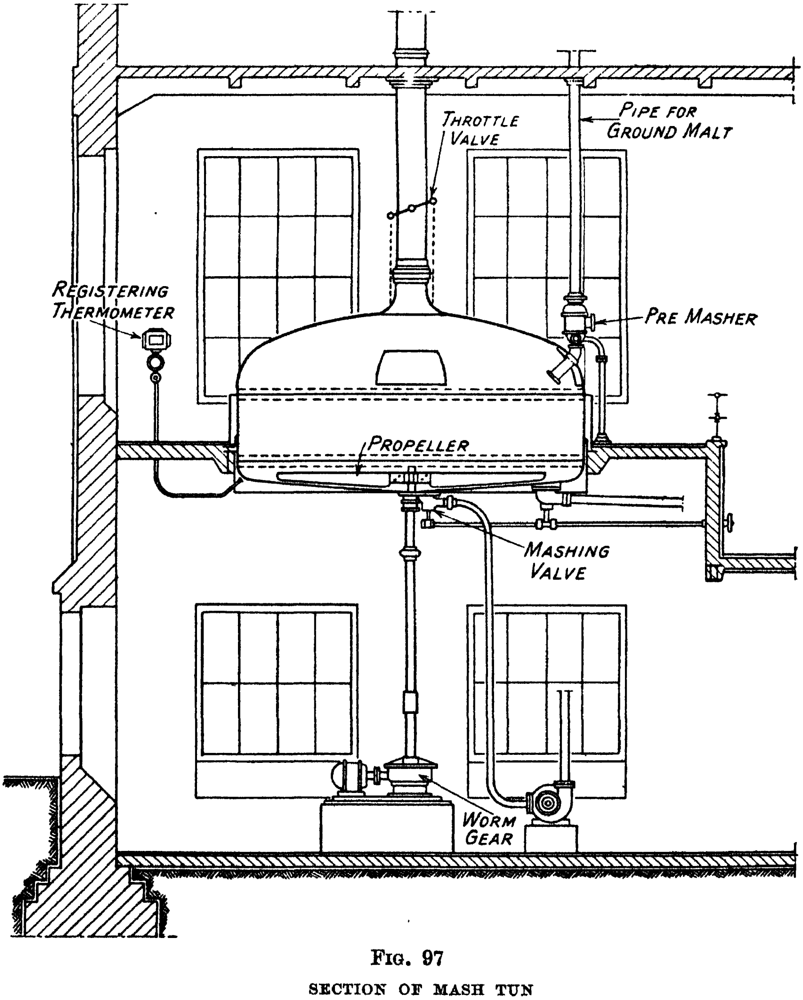

Fig. 97

SECTION OF MASH TUN

Mash copper.—A copper or iron vessel, with domed bottom and propeller designed to drive the mash upwards from the sides of the tun to the middle. It is generally heated by means of a steam jacket, but fixed or revolving coils are sometimes fitted. It is used for boiling portions of the mash and for heating liquor.

Lauter tun.—This is generally used for settling the mash, running off the wort and sparging only, the complete mash being transferred to it from the mash tun. It is sometimes, however, used for mashing. It is constructed of copper or cast iron on similar lines to Fig. 97, but fitted with rakes and heat-insulated by a copper jacket, giving an air space round the upper part of the vessel, or by lagging covered with wood or tiles. It is placed at a higher level than the wort copper, to which the wort runs by gravity. The taps are frequently fitted with saccharometers for testing the gravity of the running wort. It is desirable that the lauter tun should be of sufficient size to ensure that the depth of grains is between 12 and 18 inches, to provide an efficient filter and a large surface, so that all the worts can be collected in 2½ to 3½ hours ; 10, 20 and 30 quarter tuns (1,500–4,500 kgms.) should have diameters not less than 10, 14½ and 18 ft., respectively. A greater depth causes the goods to settle more tightly in the tun. Rapid sparging is desirable to secure maximum extract, without unpleasant flavouring substances from the husks, a point to which careful attention is given in brewing pale lager beer.

Wort copper.—This is a copper, iron or, most recently, stainless steel vessel of similar construction to the mash copper, but of sufficient capacity to boil the whole brew with hops. It is heated by steam in jacket, coils, central heater or, less frequently, by fire, oil or coal gas. Some brewers pass the steam from the boiling wort through calorifiers, in which hot liquor from the wort refrigerator is heated for mashing.

(419) The Brewhouse.

The complete plant thus consists of four vessels, the mash tun, mash copper, lauter tun and wort copper. Two sets of vessels constitute the " double brewhouse " of larger concerns. That illustrated in Fig. 99, with a capacity of 10,000 kgm., or 70 quarters of malt per brew, is also provided with two small, copper-cased, stainless steel vessels in which a biologically acidified mash can be prepared (Section 457). These are situated in front of the mash coppers and can be seen in Fig. 68, Section 376, which is from a photograph of the exterior of the brewhouse. With their lacquered copper domes and chimneys to remove all steam from the room, these vessels lend themselves admirably to artistic decorative schemes. Finished with white or coloured tiles and such structural refinements as are shown in the photographs, they form an advertisement for the brewery as well as an efficient centre of production. The pair of premashers on each mash tun of this particular installation are designed to permit, if desired, of separate mashing of husks and finer grist prepared in the malt room above. The vertical pipe into the mash copper can be used for grits, while there is a hot and cold liquor mixer at each tun.

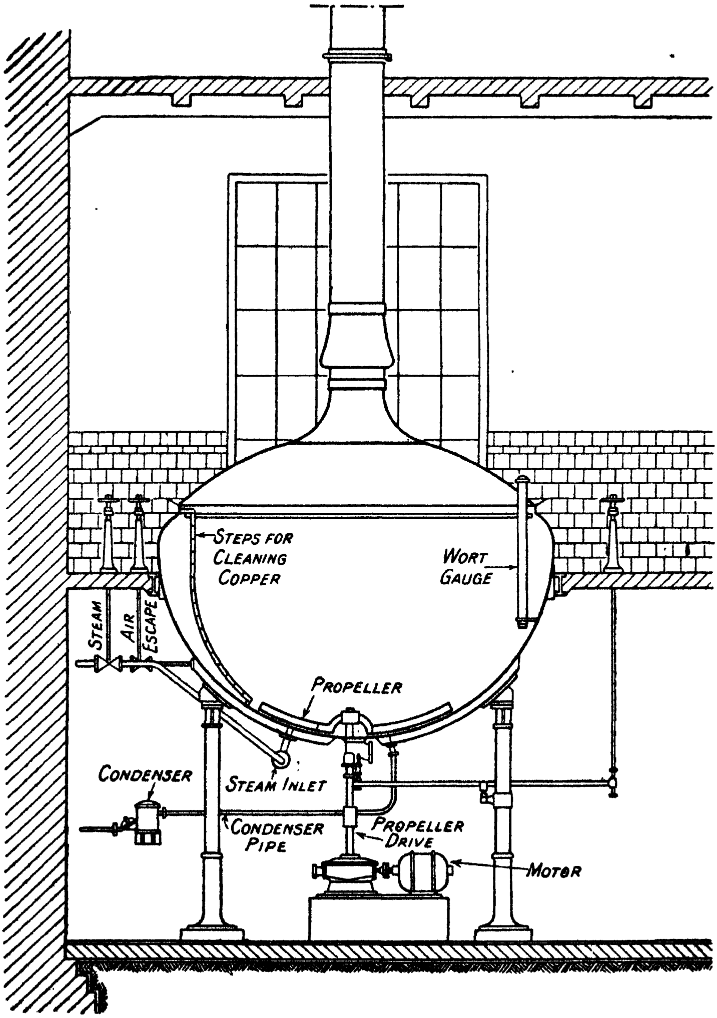

Fig. 98

SECTION OF WORT COPPER

An independent mash copper is omitted in many modern brewhouses, which comprise two sets of three vessels, as shown in Fig. 100. These each consist of a combined mash tun and mash copper, a lauter tun and wort copper. This arrangement saves space and is capable of dealing with six brews in 24 hours. The mash, made on one side, is boiled in the mash tun of the other, transferred to its own lauter tun and finally to the wort copper on its own side. A mash can follow on the other side as soon as the wort is pumped to the lauter tun. In smaller brewhouses, with a single set of three vessels, the wort copper is used for boiling the mash. In still simpler plants, the mash tun is eliminated, mashing being carried out in the lauter tun, but only one mash can be carried through in 12 hours.

The pumps and mains, below the mash room, are a most important part of lager brewhouse plant. Fig. 101 represents a typical installation. One centrifugal pump can be used for all purposes, but two are necessary for large plants, one for the mash, the other for the wort. The mash pumps are designed to avoid damage to malt husks, while sharp bends in the pipe lines and obstructions in the valves are avoided for the same reason. Another pump is, in some cases, used for removing the grains from the lauter tun. The propellers, rakes and pumps are usually driven by direct coupled motors, with controls in the room above. A closed hop strainer or montejus is almost invariably used in lager breweries to separate the spent hops from the wort and this is also placed below the mash room.

MASHING PROCESS

(420) Three-Mash Processes.

The objects of the various stages in the triple decoction process with temperatures commonly used in each are summarised in Table 162.

TABLE 162.—THREE-MASH PROCESS

	Temperatures		Objects
	C.	Fahr.	
Hydration ..	35°	95°	Mashing or "Doughing in." Solution of sugars, salts and enzymes.
1st Mash ..	50°	122°	Proteolytic action.
2nd Mash ..	65°	149°	Starch conversion.
3rd Mash ..	75°	167°	Stabilisation.

The mash rate and temperatures are varied somewhat, according to the character of the malt and the desired composition of the wort or type of beer. About 2·2 Hl. of liquor is commonly allowed for each centner of malt, corresponding to a rate of 4 barrels per quarter. As much as 2·5 to 3·0 Hl. of liquor per

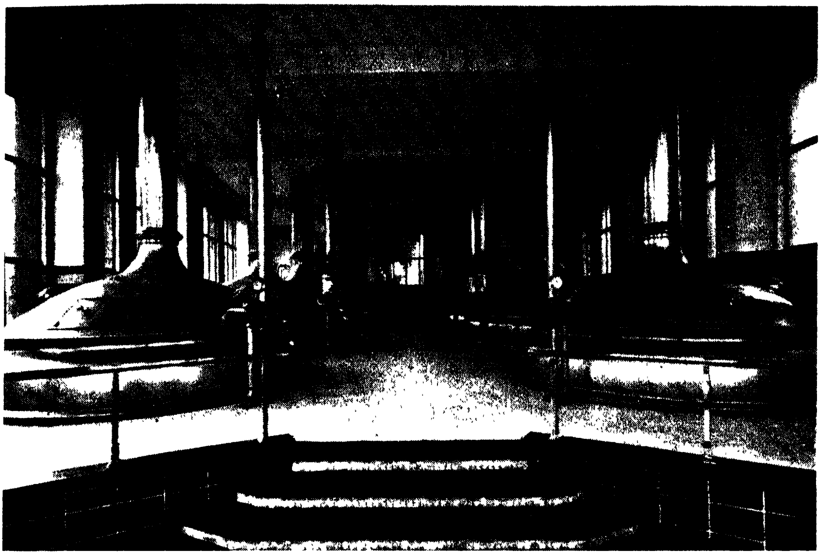

FIG. 99

DOUBLE BREWHOUSE (BRASSERIE WIELEMANS-CEUPPENS, BRUSSELS)

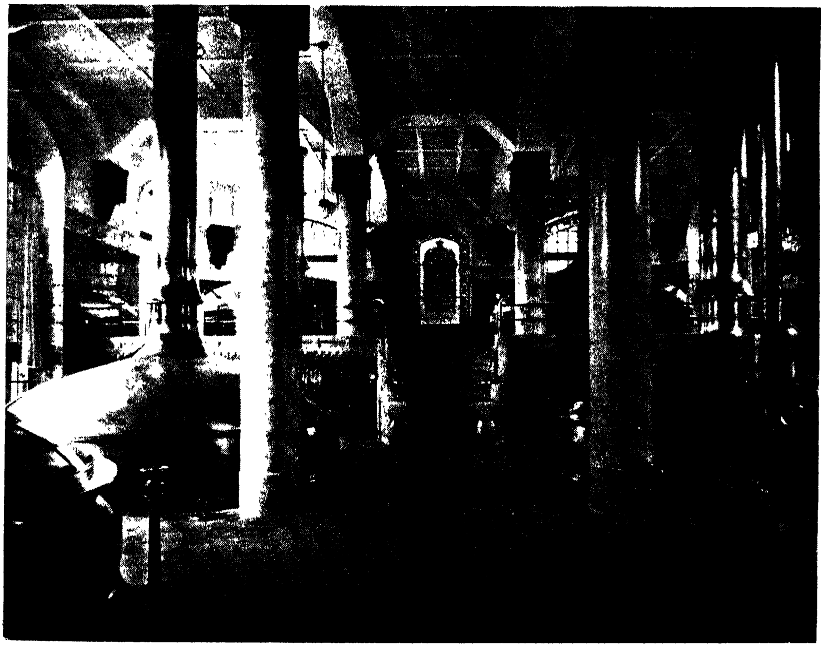

FIG. 100

DOUBLE BREWHOUSE (BRAUEREI FELDSCHLÖSSCHEN, RHEINFELDEN)

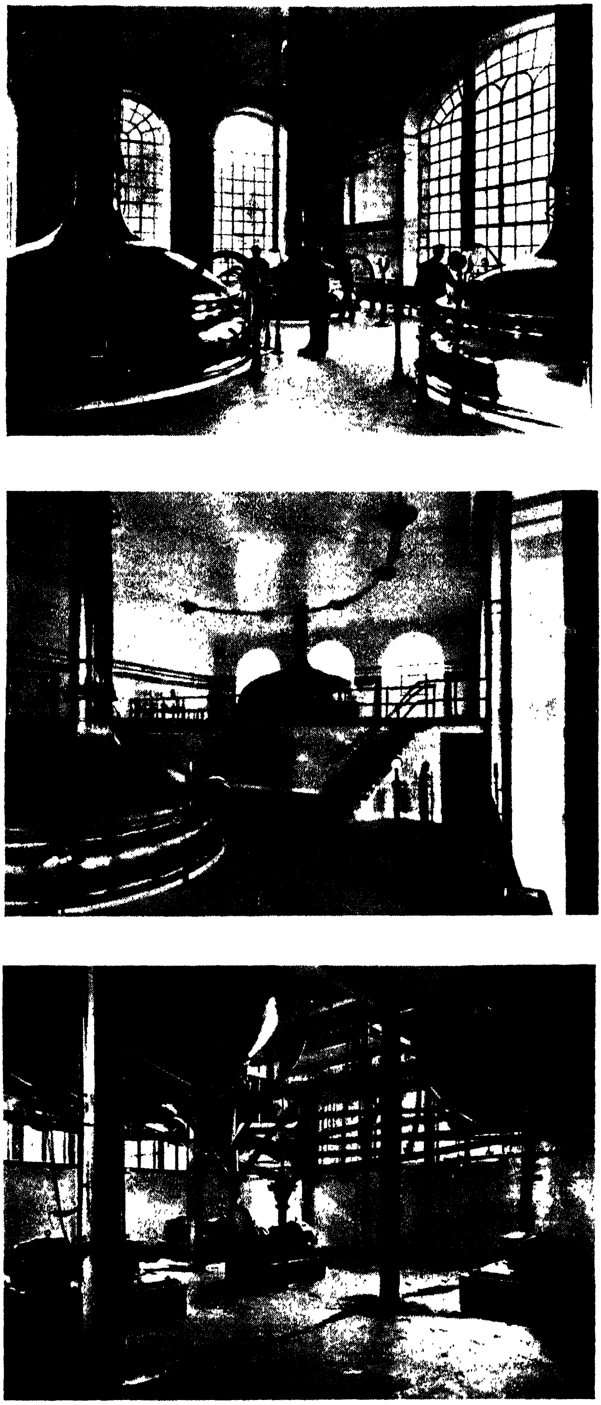

Fig. 101

BREWHOUSE PLANT (HAMBURGER BREWERY, STOCKHOLM)

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50 kgm. of malt (4½ to 5½ barrels per quarter) may be allowed for pale beers. For dark beers, a mashing rate of between 1·5 and 2 Hl. per 50 kgm. (2¾ to 3¾ barrels per quarter) is to be preferred.

The actual mash or hydration may be made with cold or warm liquor, as shown in the Table. In the former case, about two-thirds of the above-mentioned quantity of unheated liquor is used for "doughing in," while one-third is boiled in the mash copper and added to the mash, with the propeller in rapid rotation. Usually, the hot liquor is added immediately after mashing but, with poor quality or under-modified malt, it may be of advantage to allow a short rest at ordinary temperature. In rare cases the cold mash is allowed to stand 8 to 12 hours before the temperature is raised to 95° F., but this involves the danger of acidification if the temperature rises above 68° F. In ordinary circumstances, the hot liquor is pumped over slowly from the copper, to raise the temperature of the mash to between 95° and 104° F., the propeller being kept in rapid revolution meanwhile. Alternatively the mash can be made directly at 95° to 100° F., with liquor adjusted in temperature at the premasher, sufficient liquor having previously been run into the tun to cover the propeller.

One-third of the mash is then pumped over to the mash kettle and gradually heated to 149°, at which temperature it is held for about twenty minutes. It is then heated to the boiling point, again at the rate of about 1½° F. a minute, and boiled for times which may vary from ¼ hour for pale beers to ¾ hour for dark, when it is pumped back to the mash tun. The temperature of the mash should in this way be raised to between 122° and 129° F. Up to this point the process occupies about 2½ hours.

A second thick mash of about one-third the total quantity is then raised to boiling in about half an hour and boiled for half an hour as before. On being pumped back to the larger part of the mash, which has been standing meanwhile at about 122° F., the temperature of the latter is raised to between 144° and 149° F. After the goods have settled, a third, but this time a thin mash, of about one-third the total quantity in the mash tun is boiled as before in the mash copper and returned to give a temperature of between 167° and 172° F. The mash is then pumped over to the lauter tun, and after a stand of a quarter to half an hour (during which time the goods settle down in layers of different specific gravity and composition, forming a good filter bed), the worts are run off and sparging carried through.

(421) Pilsen System.

The process and temperatures, given by Jalowetz as typical of the mashing methods employed at Pilsen, are rather different from the foregoing and indicate how the triple decoction system may be varied in detail, while still retaining its salient features. Only two vessels are used, a combined mash and lauter tun and a copper. The mash is made at the rate of 6 to 6·3 hectos per 100 kg., or about 5·5 barrels per quarter, to give a first wort of 12·5% or about 1050 sp. gr. The initial rise of temperature given for each mash in Table 163 is secured by pumping an appropriate proportion of the main mash from the mash tun to the copper, where sufficient boiling liquor or boiling mash, as the case may be, had been retained to give the required increase. The underlined figures represent temperatures in the mash tun. The others are temperatures in the copper.

TABLE 163.—THREE-MASH PILSEN PROCESS

	Temperatures		Process
	C.	Fahr.	
Hydration	cold	cold	
	35°	95°	By hot liquor pumped from mash copper.
1st mash	52·5°	126·5°	By hot liquor retained in mash copper.
	62·5°	144·5°	Raise in 13 minutes.
	75°	167°	Raise in 20 minutes, saccharification 8 mins.
	boil	boil	Raise in 12 minutes. Boil 30 minutes.
	43·5°	110·5°	Return to main mash.
2nd mash	62·5°	144·5°	By hot wort retained in mash copper.
	75°	167°	Raise in 20 minutes, saccharification rest.
	boil	boil	Raise rapidly. Boil 15 minutes.
	62·5°	144·5°	Return to main mash.
3rd mash	88°	190·5°	By hot wort retained in mash copper.
	boil	boil	Raise in 12 minutes. Boil 10 minutes.
	72·5°	162·5°	Return to main mash.

(422) Mashing Temperatures.

The triple decoction system emphasises the reactions which occur at the different salient temperatures and makes use of them all to the best advantage. Below 100° F., enzyme activity is slight, but the enzymes are extracted from the malt, together with soluble carbohydrates and nitrogenous substances, while the salts in the liquor interact with the phosphates and organic acids extracted from the malt. At the same time there may be a slight rise in acidity, due to bacterial action, and the insoluble starch, proteins and other constituents of the malt are thoroughly soaked.

At the second stage, between 122° and 129° F., the proteolytic enzymes are particularly active and the major part of what protein digestion occurs in the mash tun is carried through at this temperature. The mash is subjected to this protein rest for between 1½ and 2 hours. Saccharification occurs most effectively during the third stand between 144° and 149° F., when the carbohydrates are converted to the extent desired, by suitable variation of the temperature. The composition of the wort is fixed when the boiled clear mash is pumped back to the mash tun, giving a temperature between 167° and 172° F., which effectively stops the saccharifying action of the diastase, but may leave some of the liquefying activity in being. To this point the process may take between 5 and 6 hours, according to the duration of the boils and stands.

The changes occurring in the mash tun are thus, in the main, enzymic, while those brought about by boiling have largely a physical character. The slow rise in temperature of the thick mashes is intended to allow them to pass slowly through the critical stages and, particularly, to permit of saccharification during the short stand at 149° F., in the first mash. The subsequent slow rise to the boiling point assists in the softening of under-modified malt and the liquefaction of its starch, but the essential parts of the two thick mashes are the boiling periods. During this the starch and protein-containing cells of the malt are softened, broken down by physical means and brought into a condition ready for conversion of the protein and starch in the succeeding periods of rest. The starch is gelatinised and there is coagulation of part of the protein, resulting in early removal of excess from the sphere of enzyme action.

(423) Modifications in Three-Mash Process.

The diagram of a three-mash process in Fig. 102 will show how readily alterations can be made at any stage to suit the properties of the malt and character of the beer or for economy in time, fuel and plant. The complete process is most suitable for dark beers, made from malt with low diastatic activity or for under-modified malts. It has already been noted that a protein rest is more necessary with lager malts than with English-made malts in which proteolysis is carried further in the maltings. Dark lager malts usually contain a higher proportion of protein degradation products than pale and consequently may not require a long protein rest. Dark malt mashes are generally boiled for longer periods than the pale, as the flavours that are extracted from the husks and the extra colour secured may be an advantage with dark beers. The method is not so suitable for highly diastatic or fully modified malts and pale beer brewing.

The very full conversion may yield a too readily fermentable wort, while the delicate flavour and pale colour are detrimentally affected. Modifications can be made in two ways to restrict saccharification. A greater quantity of the mash can be boiled, in order to raise the saccharification temperature reached when it is pumped back to the main mash, or part of the boiled mash may be left in the mash copper and the next portion to be boiled added to it. By this means the active saccharification temperatures are more rapidly passed over, than when the mash is heated in the usual way. Variations are, however, usually made by reducing the number of boils.

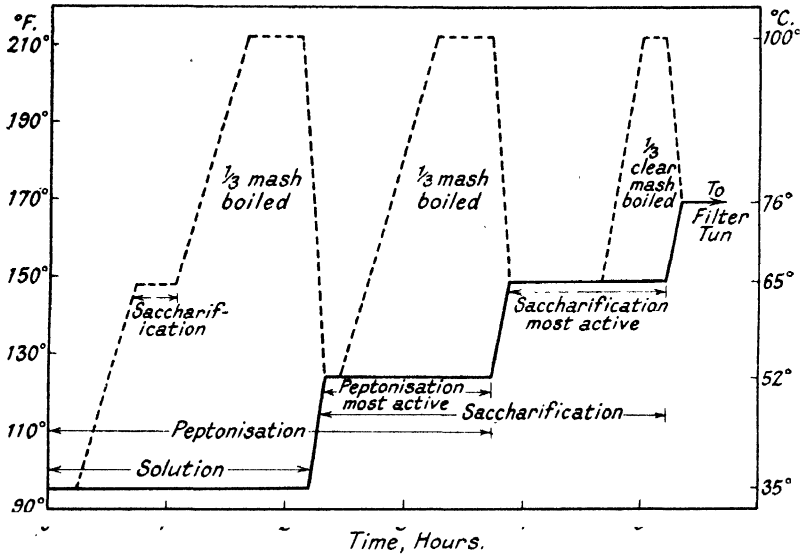

FIG. 102

DIAGRAM OF THREE-MASH PROCESS

(424) Double Decoction or Two-Mash Process.

This is more flexible than the three-mash system and better adapted to the more highly diastatic pale malts. Variations can readily be made according to the character of the malt and the wort composition desired. Thus the mash may be made with cold water and then brought up to 100° or 122° F., with boiling liquor, by steam coils, or, if preferred, mashed directly at the higher temperature. The protein rest may be given, abridged or omitted previously to bringing the mash up to the conversion temperature between 149° and 158° F., by means of boiled thick mash. A second thick mash is made to give the final temperature of 167° or rather higher. It is desirable to give a protein rest of 20 to 45 minutes with coarse, under-modified malts but, with finer, more fully modified malts, this can be omitted altogether. The saccharification is also adjusted according to the modification and diastatic activity of the malt, with due regard to the limitation of attenuation brought about by raising the temperature and consequent restriction of the maltose percentage. In the mash illustrated in Fig. 103, intended for a fairly well modified malt, the peptonisation temperature is passed over and a rather high saccharification temperature given as well.

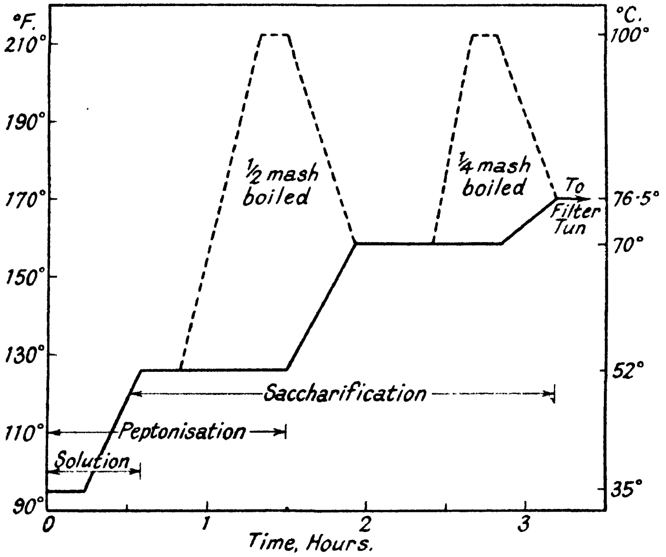

FIG. 103

DIAGRAM OF TWO-MASH PROCESS

(425) Rapid Mashing Process and Single Decoction Processes.

Among the special modifications of the double decoction system is the *Hochkurzmaischverfahren* or high-temperature, short mashing process proposed by Windisch.[1] As first described, this process included two boils, but the time of boiling was much reduced as shown in Fig. 104. A comparatively thick mash is made at about 145° F. and, as soon as the quantity in the tun permits, sufficient is pumped over to the mash copper, raised rapidly to the boil and returned to the mash tun immediately the mash is all in, raising its temperature to 158°. After saccharification is completed, in about half an hour or an hour, a second thick mash is raised rapidly to the boiling point and after boiling for 5 or 10 minutes returned to the mash, raising the temperature of the latter to 167°. The mashing time is in this way reduced to about 2 hours, from start to attainment of the final temperature, and a wort produced in which the nitrogen compounds depend in the main on those rendered soluble during malting. The idea governing the rapid increase of temperature to that required for saccharification was that head depended mainly on nitrogen compounds of high molecular complexity and that it was only with under-modified malts that the protein rest of the three-mash process was desirable. The process has proved very successful and is used in a number of breweries for pale beers, but it is essential that the malt should be well modified in order to obtain a sufficiently fermentable wort at the high saccharification temperatures. It is claimed that the quality of the beer is improved by reduced extraction from the husks in the shortened boils.

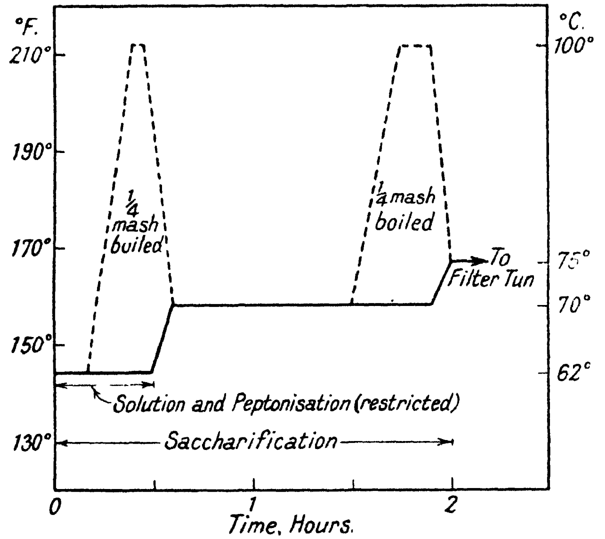

FIG. 104

DIAGRAM OF RAPID, HIGH TEMPERATURE PROCESS

The second boil may be replaced by use of the coils for raising the mash to its final temperature of 167° or 170° F. in which case it becomes a single decoction process. The first mash is then generally boiled for 10 or 15 minutes. If it is desired to increase proteolysis, the original mash may be allowed to stand at 122° for 15 minutes before boiling the first mash, or it may even be mashed in at 95°, when it would be necessary to boil about three-quarters of the mash to give the saccharification temperature of 158° F.

In other single-mash processes, the thinner part of the mash may be withdrawn, either after the first mash has settled down or after the mash at saccharification temperature has settled, and held back to saccharify the boiled mash. Thus the whole of the mash made at 122° may be brought up to saccharification temperature in the mash copper with the propeller in action and the diastatic liquid withdrawn after settling. The thick part of the mash may then be slowly raised to boiling and kept in ebullition for an hour, after which water is added to bring the temperature back to, say, 158° and held-over liquid returned. The diastatic liquid may be drawn from the first low temperature mash, if it is desired to pass rapidly over the saccharification temperature and get a dextrinous wort of low fermentability. The thick part of the mash would then be raised rapidly to boiling and cooled back to 160° or rapidly raised to that temperature without boiling, the diastatic liquor then being returned for saccharification and the temperature raised to 170° when conversion is complete.

(426) Flexibility of Decoction Processes.

It is clear that great variations are possible in the decoction process of mashing. The temperatures may be changed at will and the length of stand at any of them varied. One, two or three mashes may be boiled for longer or shorter periods or the temperatures may be raised by the use of steam coil and propeller. The advantages and defects of the various processes have been argued many times. Extract, time required, rapidity of sparging and wort composition must all be considered. The three-mash process takes longer than the others, but fulfils all other requirements and many still hold that it is the safest, since it deals adequately with under-modified malts, but the two-mash processes are equally as successful for pale beers. The quantity of mash boiled may have a considerable influence on the conversion of under-modified malts, but the difference in quantity is small between the three- and two-mash processes. The amount boiled in the single mash process is less and, consequently, more fully modified malts must be used. A considerable increase in proteolysis can be secured in a single mash by holding the first mash at 120°–130° for 15 minutes before raising it to 158°, with very little sacrifice of speed.

(427) Decoction Mash with Raw Grain.

In European countries in which the use of raw grain is permitted, a modification of the two-mash process is usually adopted, when grits are used in proportion of 10 to 20% of the grist. Equal quantities of grits and malt are, for example, mashed in the mash copper at a rate of about 4 to 4½ barrels per quarter of 3 cwt. This is raised slowly to 122° F., and allowed to stand at this temperature for half an hour, then slowly heated to a saccharification temperature of 158°–167° F. at which it is kept for half an hour. The mash is then boiled until the grits are completely gelatinised. This may require from 20 to 40 minutes, according to the fineness of the grits. When this mash begins to boil, the remainder of the malt is mashed in the mash tun at the rate of 1½ barrels per quarter and given a protein stand while the grits are boiling. The boiled grits take the place of the first mash and, when pumped to the main mash, raise it to the desired saccharification temperature by correct adjustment of the quantities. In some breweries, maize or rice grits are used with a three-mash process, the grits taking the place of the first boil. The temperatures may be 95°, 126°, 145° and 167° F.

(428) Sparging.

Sparging is carried out in the same manner as in the infusion system. The essential function of the lauter tun is that of a strainer. The space beneath the false bottom is filled with liquor before the mash is pumped over. A stand of ¼ to ½ hour is then allowed for the grains to settle into an efficient filtering bed, consisting of layers depending on the specific gravity of their constituents. The heaviest husks settle at the bottom, followed by lighter husks and then a more compact mass of finer material and protein sludge. The appearance of a " marbled break " in the overlying wort is a good sign that a satisfactory filter bed has been formed. This layer of wort is usual in decoction mashes and not a sign of defective operations, as it is in the thicker infusion mash. The thickness of the bed of grains should not be less than 12 inches or greater than about 18 inches in order that filtration may be effectively and rapidly carried out.

DOUBLE-MASH SYSTEM

(429) The Double Mash.

This is a modified infusion system, developed in America for pale lager beers, but also used for top fermentation ales. It is typical of methods employed with high percentages of rice or maize grits, of which 25 to 40% are commonly used with highly diastatic malt, made from Manchuria type barley. The mashing process is illustrated by the diagram, Fig. 105, and the plant by a photograph of the experimental brewery of the Wahl-Henius Institute, Chicago, reproduced in Fig. 106. The process is characterised by two separate mashes, the main mash of most of the malt, and the grits mash with a small quantity of malt to facilitate liquefaction. The temperature of the former and the quantities of the two mashes are so adjusted that the combined mash shall be brought up to the selected saccharification temperature when the boiling gelatinised grits mash is added to the malt mash.

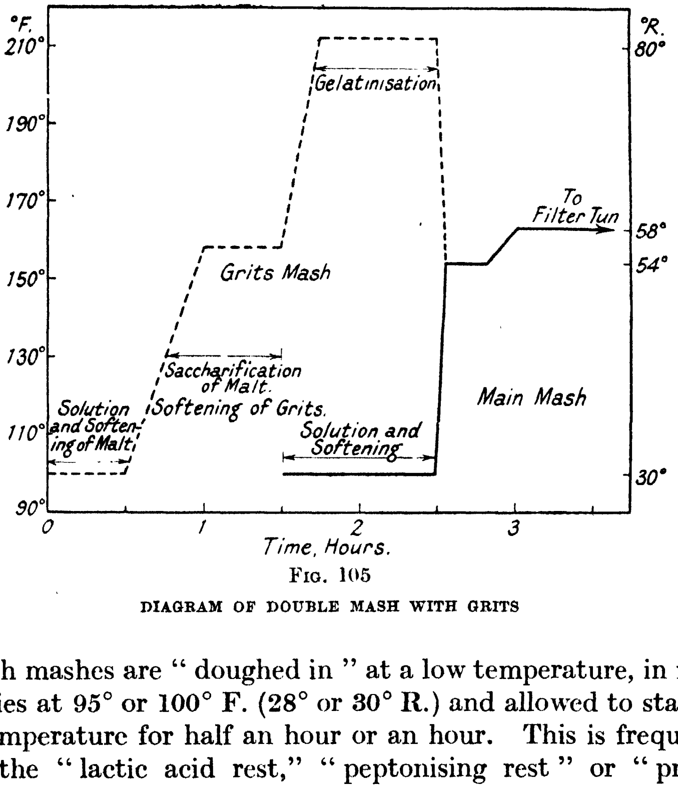

FIG. 105

DIAGRAM OF DOUBLE MASH WITH GRITS

Both mashes are " doughed in " at a low temperature, in many breweries at 95° or 100° F. (28° or 30° R.) and allowed to stand at that temperature for half an hour or an hour. This is frequently called the " lactic acid rest," " peptonising rest " or " protein rest," but these are rather misnomers. The time is too short for anything more than a slight production of lactic acid by bacteria and the fall in p_H from its original value of 5·6–5·8, (which are normal figures with liquors that contain 10–12 parts per 100,000 of carbonates and little or no gypsum, or rather lower values if "Burtonizing" is adopted), is very slight. Enzymes are extracted, phosphatases and proteases commence to function, but the temperatures are rather low for the considerable activation of proteolytic enzymes implied by peptonising or protein rest. Inorganic phosphates may be liberated to some extent and tend to reduce the p_H value, but one of the processes described in Sections 455 to 459 must be used, if it is to be brought anywhere near 5·3 or 5·2 to increase enzyme action. The true protein rest occurs at temperatures between 113° and 122° F. (36°–40° R.)

which are adopted in some breweries. The saccharogenic or β-amylase is active at these temperatures but, in its turn, functions better at between 133° and 140° F. (45°–48° R.), when the dextrinogenic or α-amylase comes into the picture and assists conversion. Saccharification is restricted to some extent at temperatures above 145° F. (50° R.) and those above 149° F. (52° R.) are selected for that reason and to activate the α-amylase, without destroying the saccharifying activity of β-amylase too rapidly.

The Manchuria malt is very pale dried and generally has a diastatic activity of 80°–120° Lintner. Its proteolytic activity is also high. The raw grain may be broken rice or maize grits. Modifications of the process are necessary if flakes or the very rapidly gelatinising "refined grits" are employed. The times for starting the cereal mash and the malt mash are arranged so that the latter shall have had the required low temperature stand for half an hour or an hour by the time the grits are gelatinised and ready to pump over to it. This may mean that the grits are mashed with one-third their weight of malt, one and a half hour before the malt mash is made. The diagram shows the grits held for half an hour at 95° F. and then raised slowly to 158° F., at which they are held for half an hour while the accompanying malt is saccharified. This process facilitates the subsequent gelatinisation of the grits, which is secured by raising the temperature more rapidly to boiling and keeping the mash in active ebullition until the grits are completely softened, as judged by pressing a little between the finger and thumb. This process is carried out in the cooker, the right-hand vessel of the two in the centre of the photograph, Fig. 106. In the meantime, the bulk of the malt has been mashed at 95° F. in the left-hand vessel and allowed to stand for half an hour or an hour, by which time the grits should be gelatinised. It is best to mix the two mashes in a third tank, shown behind the other two, but sometimes they are mixed in the mash tun or lauter tun, in the latter of which the mash may have been made in the absence of a separate mash tun. The grits must be pumped over rapidly to avoid the risk of cooling and gelatinisation in the pump or main. Thorough mixing of the two mashes is assured by means of an efficient propeller. The temperature after mixing is shown in the diagram as 154° F. (54° R.), a very usual saccharification temperature. This is held for a quarter of an hour and then raised to 163° F. (58° R.) by hot liquor or steam injection. The mash is finally transferred to the lauter tun, shown below the liquor tanks in Fig. 106. If a separate mash tun is not available and the mash is made in the lauter tun, the slots in the false bottom must be

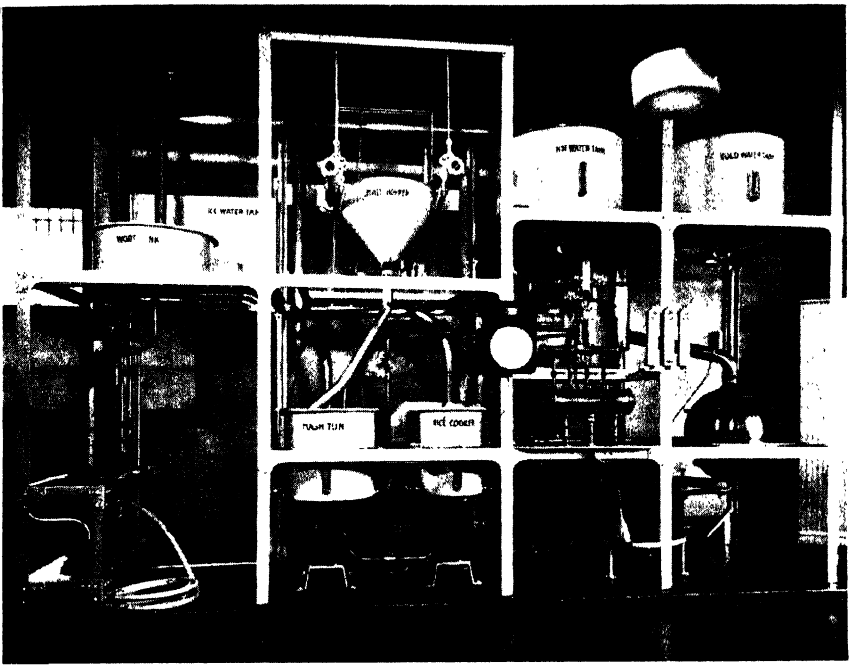

FIG. 106
EXPERIMENTAL BREWING PLANT (WAHL-HENIUS INSTITUTE, CHICAGO)

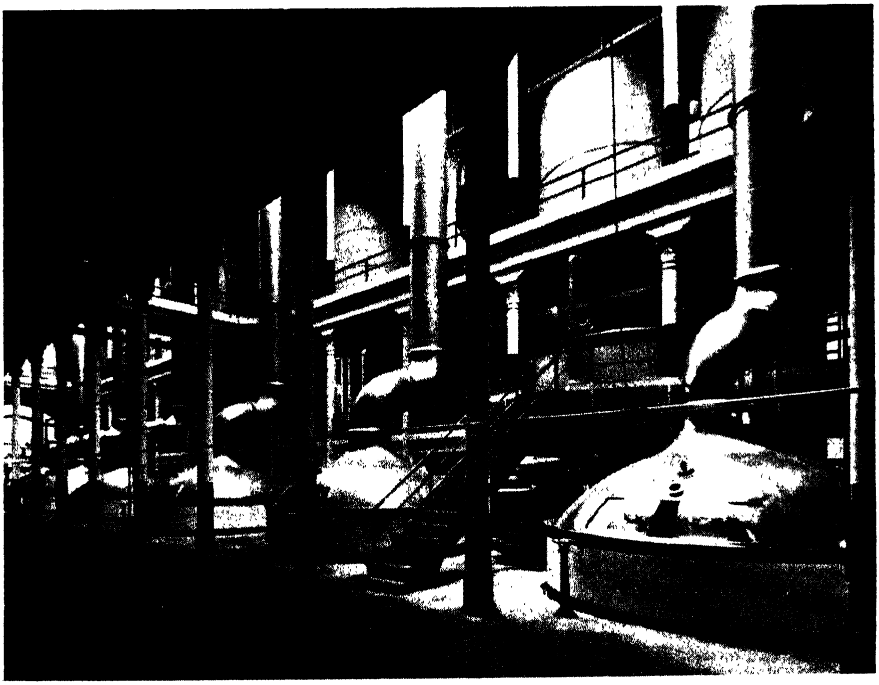

FIG. 107
KETTLE FLOOR OF AN AMERICAN BREWERY (PREMIER-PABST CORPORATION, MILWAUKEE)

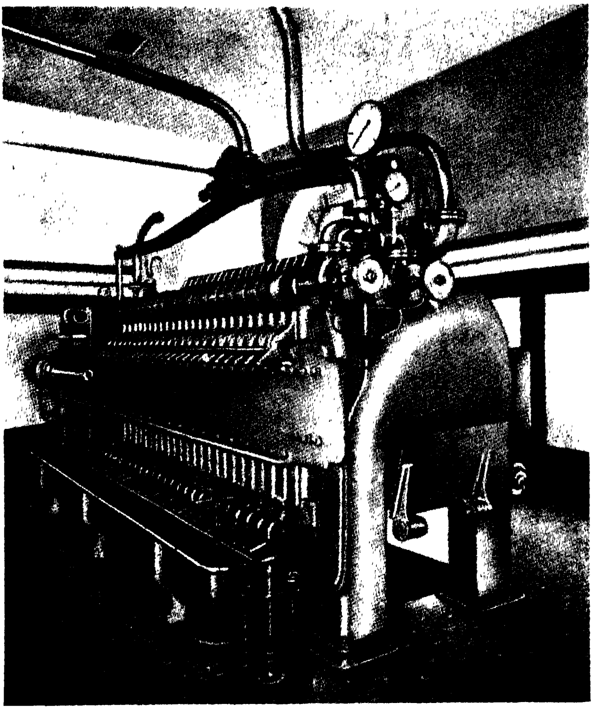

Fig. 108
MASH FILTER (HAMBURGER BREWERY, STOCKHOLM)

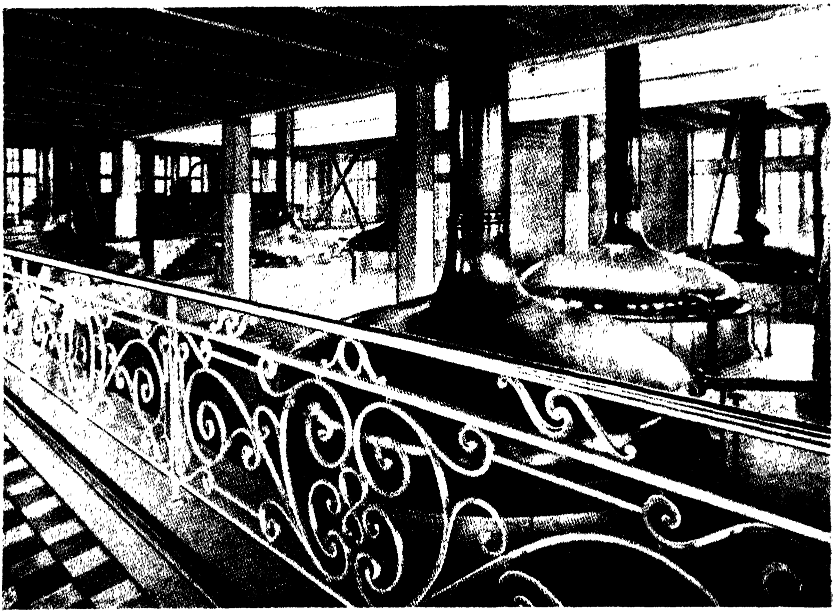

Fig. 109
DOUBLE BREWHOUSE WITH MASH FILTERS
(BRASSERIES DE CHAMPIGNEULLES, FRANCE)

cleared with a flush of hot liquor to facilitate running off to the copper or kettle, shown at the right of the photograph. The vessel on the left is a wort receiver, serving the refrigerator.

A photograph of a large plant based on this system is given in Fig. 107. Three grist storage tanks are in the foreground on the balcony. The two next to them are hot liquor tanks, followed by a mash tun and cereal cooker. The malt mash and cereal mash are pumped together to a tank below on the mezzanine floor and, after a short stand, the completed mash is pumped either to a lauter tun or mash filter. Two lauter tuns are situated behind the first and second kettles, but only the first of them is visible. Behind kettles Nos. 3, 4 and 5 are the mash filters, two of which have replaced three lauter tuns, and behind No. 6 is another lauter tun. The kettles or coppers in the foreground are each for 300 American barrels (215 British barrels) and are heated by revolving steam coils. The lauter tuns and mash filters are connected to a header, so that the wort may be discharged into any kettle at will. Each pair of mash filters has a capacity of 14,000 lb. of grist, the quantity used for each mash.

In some breweries, the main mash is made in a cylindrical boiler-shaped mashing vessel, fitted with paddles revolving round a central horizontal shaft. These mix the mash efficiently and not too violently. The vessel may be fitted with coils for adjusting temperatures with steam or cold liquor, or with jets for live steam.

One or two per cent. more extract can frequently be obtained from rice or maize grits by boiling under pressure in a closed vessel. Billing's Converter was an early form of pressure cooker, in which agitation of the mash was effected by vertical paddles, revolving round a horizontal shaft, and which could be heated either by direct steam or steam jacket. Frisch devised a horizontal cooker, fitted with similar paddles and better adapted to deal with coarse grits, which were boiled under a pressure of 60 lb. When they were thoroughly liquefied, the steam was blown off, a vacuum created and the malt mashed into the cooled cereal mash in the converter itself. The Rasch pressure cooker is a vertical vessel, fitted with horizontal shaft and vertical paddles, heated with live steam and fitted with pressure gauge and thermometer. The cereal mash is made in this in the usual manner, with one-third malt, about an hour before the main mash, or at the proper time to be ready to transfer to the main mash at the end of whatever low temperature stand is decided on for the latter. After the usual stand at 100° F., the temperature is slowly raised by means of live steam to 145° F. (50° R.) and, after 15 minutes for saccharification of the malt and a preliminary action on the grits, the steam is increased until the pressure reached is 15–20 lb., giving temperatures of 240°–255° F., at which gelatinisation is very rapid and complete. The pressure in the converter is sufficient to force the grits over into the main mash. Allowance must be made for 5 barrels or so of condense from the steam, when calculating the quantity of liquor for the grits mash, to give the desired saccharification temperature in the main mash, after mixing the gelatinised grits with it. Vertical pressure cookers are now made with cone-shaped bottoms, propellers and properly spaced baffles to ensure thorough mixing. They are heated by means of steam jackets, or with live steam from silent, brass nozzles fitted with brass check valves.

If it is desired to reduce the plant to two vessels, combined mash and lauter tun and copper for boiling grits and wort, it is convenient to provide the former with 6 or 8 steam jets with their nozzles turned in the direction of revolution of the rakes. Clean raw steam, free from oil, is quite suitable for raising the temperature of the mash.

MASH FILTERS

(430) Use and Construction of Mash Filters.

The functions of the lauter tun can be performed, with economy in space and time, by a filter press, fitted with cloths as filtration supports. Use of the filter in place of the tun is frequently decided by output requirements, since this can be increased by its use to the extent of at least one more brew in 24 hours, without any

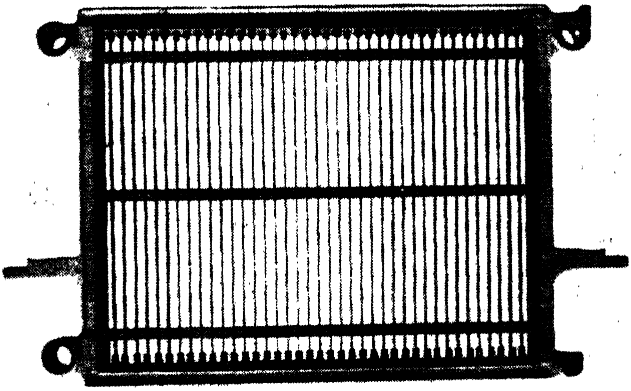

FIG. 110
MASH FILTER GRID

other alteration in the plant. The mash filter can be assembled, used and prepared for the next brew in 2½ to 4½ hours, according to its size and the length of the brew, though it is best to allow

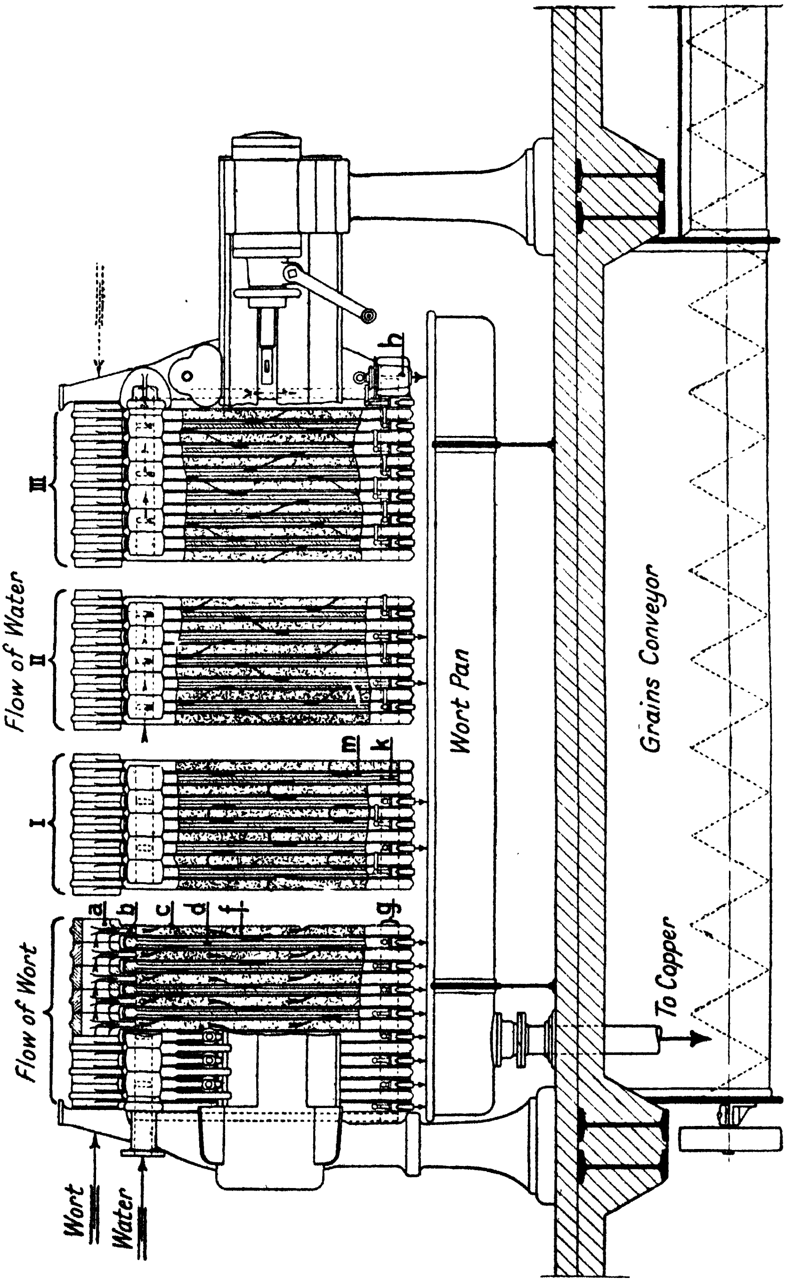

Fig. 111
DIAGRAM OF MASH FILTER

10–12 hours for 2 brews with one filter. Filters are particularly useful when high percentages of raw grain are used, as there is little liability to set mashes and less sparge liquor is required. With less completely modified malt, the filter press usually gives a higher extract than the tun, but its initial cost is greater and the cost of cloths and their washing are disadvantages.

In some filter presses a very solidly constructed framework carries frames alternating with grids, Fig. 110. The frames are each surmounted by a section of the inlet channel, so constructed as to form a continuous tube for the mash from end to end of the filter when the frames are assembled. There is a large opening connecting each of these tubular sections to the frame below. A bronze grid is placed between each frame and covered with a cloth before the complete assembly is forced together by means of a screw, which may be operated electrically or by hydraulic power. Water-tight contact of grids and frames is assured by rubber jointings. Each of the frames is furnished with liquor inlets and outlets for the wort, as shown in the figure of the grid. These form continuous mains along the sides of the assembled filter. The outlets may be connected to independent cocks or form a single wort main.

In other mash filters, the place of the grid is taken by plates against which the cloths are supported, as in the diagram of the filter in Fig. 111. Free flow of wort or water is assured by horizontal depressions at the top and bottom of the plates and vertical ribs over the greater part of its surface. The mode of operation and course of flow of both wort and sparge liquor are shown in the diagram. The plates or grids when assembled with cloths covering both sides, convert each of the frames into an independent mash chamber 2½ inches wide. When these are filled with mash, either by pumping from the mash tun or by direct flow, each cake of mash corresponds in miniature with the goods in a lauter tun, but arranged vertically instead of horizontally and offering a much greater total surface to the washing liquor passing through from the cloth covering one side to that on the other.

(431) Operation of Mash Filters.

Wort runs from the goods in each chamber through all the taps, but alternate taps are closed for sparging. The extracting liquor can enter the plates or grids either by the top or bottom channel. These serve alternate plates from which the liquor must pass through the cloth and mash, to pass out through the other cloth before reaching the run-off tap or main. The liquor can be run alternately from the top or bottom or through the mash inlet, in which case it passes out at both sides of each cake of mash. The pressure required may vary from 0·3 atm. for new cloths to

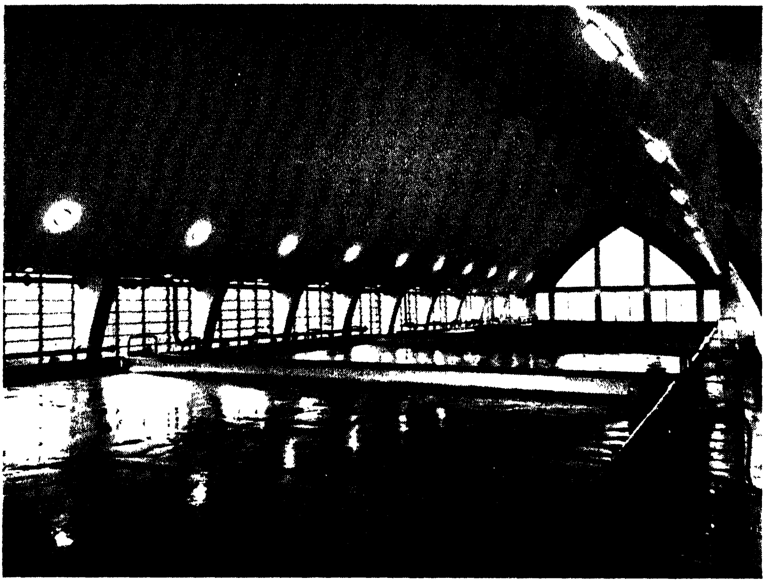

Fig. 77
STAINLESS STEEL COOLERS (TUBORG BREWERY, COPENHAGEN)

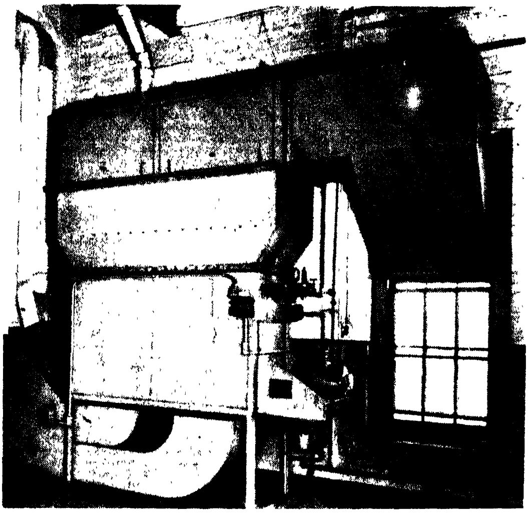

Fig. 78
AIR CONDITIONING UNIT OF REFRIGERATOR ROOM
(SCHREIBER BREWING CO., BUFFALO, N.Y.)

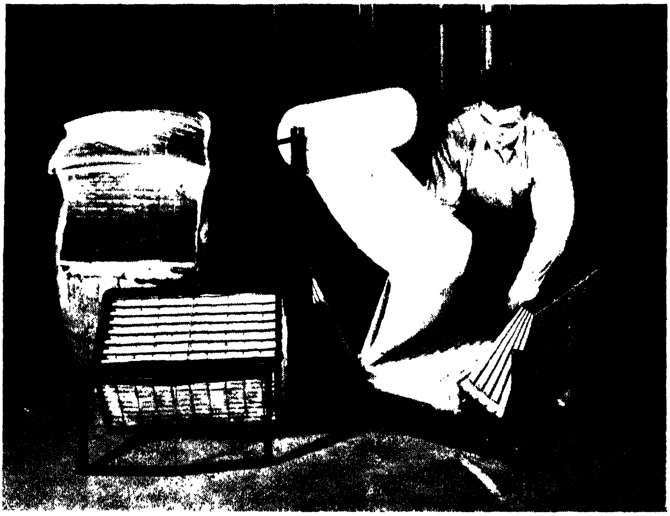

Fig. 79

ABSORBENT COTTON FILTER

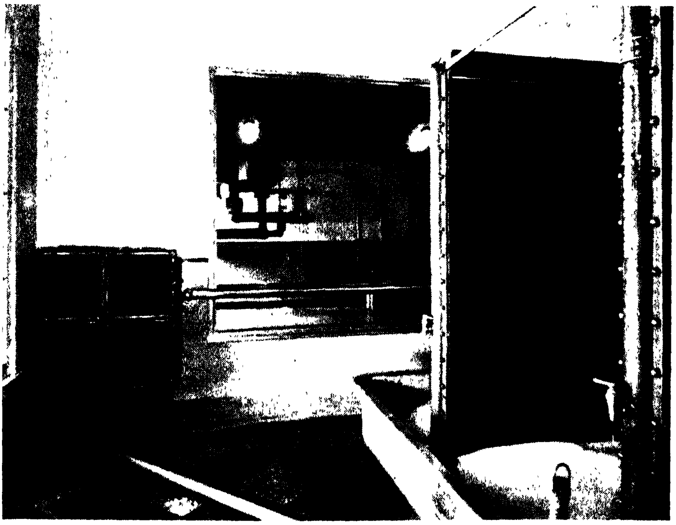

Fig. 80

VISCOUS TYPE FILTER

Air Filters for Refrigerating Room
(SCHREIBER BREWING CO., BUFFALO, N.Y.)

0·8 atm. for old cloths and is obtained either from a pump or tank. A mash filter is shown in use in Fig. 108.

Fig. 109 is from a photograph of a large double brewhouse with two complete sets of vessels and two filters in place of lauter tuns. Each filter is capable of handling 60 quarters (9,000 Kgs.) of mash. The mash tun used with a filter has a domed bottom and is fitted with a propeller, so that the mash can be thoroughly mixed when it is pumped over to the filter, evenly filling the chambers with the overflow open. The filter is filled with water at about 120° F., for a final rinsing and warming before use and it is usual to isolate one or two chambers to allow for variation in the quantity of the mash and fill them if required. Filtration is not satisfactory if the chambers are filled too loosely and is slow if they are packed too tightly. High extract and rapid filtration also depend on suitable grist. Excess of flour chokes the cloths and impedes the flow of wort. Each cake of mash must be porous and even. For this reason the husks must not be pulverised, although the grind may be considerably finer than for a lauter tun, but very finely ground grist can only be used with new cloths. As they get older a coarser grist should be used. After a few minutes' rest the taps are opened and the wort should commence to run freely. If not brilliant, it may be recirculated.

When sparging is finished the grains are allowed to drain for 5 minutes. The filter is opened by unscrewing the movable back plate sufficiently to get the cloths out and allow the grains to fall into the trough beneath, from which they are mechanically conveyed for disposal. The cloths are washed, first in warm and then rinsed in cold water. Suitable cloths are of great importance for rapid and efficient filtration. If too thin they wear badly and if too thick they delay the process. They should last for 100 mashes, but this depends on the treatment they receive and the water in which they are washed. Carbonates in the liquor deposit in the cloths and necessitate frequent washing in acidified liquor, in order to keep them soft. Centrifugal machines are used for washing, but drying may be unnecessary when the brews follow in rapid sequence.

(432) Summary.

The essential difference between the infusion and decoction mashing processes lies in the boiling to which part of the mash is subjected in the latter. Boiling has two opposing effects. It softens the malt and thereby renders its resistant constituents more readily convertible, but it destroys the enzymes present in it. The boiled mash provides a convenient means for raising the temperature of the main mash in stages most appropriate to successive extraction of soluble constituents and enzymes, activation of proteolytic enzymes and saccharification. Since the temperature changes depend on that of the main mash and on the quantity of boiling mash added, the decoction system permits of a wide range of variation at each stage of the process. Full advantage is taken of this, the details depending on the nature of the malt used and the character of the beer brewed. These must first be considered, in order that the reasons for the various mashing operations may be appreciated.

Although pale lager malt should be reasonably friable and tender, the breakdown of starch and proteins is not carried so far as in malt intended for an infusion mash. The kilning is carried out so that the enzymic activity is reduced comparatively little, while the colour is kept as low as possible, commensurate with the flavour required. Dark malt is entirely different in that a greater starch conversion is permitted and a more far-reaching proteolysis encouraged during the early kilning period. Later, with the development of colour and flavour, there is much greater restriction of diastatic activity. The pale beers are comparatively dry and fully attenuated, though they are not usually fermented so far as top fermentation beers. They are delicate in flavour and must be very pale. The dark beers are not so fully attenuated, and are very sweet and full in flavour.

Pale malt thus differs from dark in that it contains a smaller percentage of ready-formed soluble carbohydrates and nitrogenous substances, but it is endowed with a much greater enzymic activity. In both cases, it is necessary to encourage the production of maltose from the starch, in order to obtain the required attenuation. This is the case with pale malt because the starch is somewhat resistant and, with dark malt, on account of the low enzymic activity. Active proteolysis is also desirable in the mash, to produce yeast nutrients and sufficient of the intermediate products of protein breakdown for the required body and head-retention. For these reasons, the coaxing effects of the decoction system are particularly suitable for lager beer production.

The quantity of mash boiled and the time of boiling must, however, be carefully controlled, according to the beer produced. It tends to extract colour and harsh flavours from malt husks and must, consequently, be restricted for pale beers, but a long boil may even be advantageous for dark beers. Considerations of this nature have led to the popularity of the three-mash process for dark beers, and the preference of many brewers for a two-mash process or a single decoction system for pale beers. For the same reason, many brewers object to husky six-rowed malts for pale beers. When they are used, the mashing process would be modified to limit the extraction of the husks.

The system of raising the mashing temperature in stages makes it possible very conveniently to adapt decoction processes to the use of grits. The American mashing process has been developed on these lines, so that the best results may be obtained from malts which contain a high percentage of nitrogen, with a high percentage of grits, which yield no soluble nitrogen, but give a very high extract with little colour. The main mash is not boiled, the temperature rise being obtained from the boiling, gelatinised grits, so that deleterious extraction of the husks of the six-rowed malts is avoided. The mash can also be originally made at a low temperature to encourage the proteolysis necessary with the materials available.

REFERENCE

1. W. WINDISCH, *Woch. Brau.*, 1897, **14**, 21 ; *J. Inst. Brew.*, 1897, **3**, **146**.

CHAPTER XXV

THE INFLUENCE OF MASHING CONDITIONS ON WORT COMPOSITION

(433) Factors operating in the Mash.

The most important factors producing variations in the composition of wort include :

A. Grist	(1)	Composition
	(2)	Enzymes
	(3)	Physical condition and preparation
B. Liquor	(4)	Composition
C. Operative	(5)	Temperature
	(6)	Reaction, p_H
	(7)	Mashing process
	(8)	Mashing rate
	(9)	Duration of mash
D. Sparging	(10)	Method, liquor and temperature.

The influence of each of these factors on the extract obtained from the malt, on the apparent maltose content and fermentability of the wort, on the nature of its nitrogenous constituents and extent of proteolysis may be studied by analysis of the worts from laboratory mashes. The results are capable of affording a considerable amount of information on the principles of mashing and can be very helpful in practice, when circumstances call for decisions in dealing with changes in materials or defective fermentations. The analytical methods described in Sections 407 to 415 are adequate for this purpose. The actual nature of the carbohydrate and nitrogenous constituents of wort may be left for subsequent consideration (Chapter XXVII).

THE GRIST

(434) Composition of the Grist.

The composition of the grist is obviously of first importance in determining that of the wort and the relation between them is so well defined, under given mashing conditions, that it is possible to calculate that of the wort, with very close approximation.

The figures in Table 164 will, for example, be found to apply very closely to the composition of the Institute of Brewing extract wort from normally modified English and Californian malts with the stated nitrogen content and a thousand-corn weight of 35 grams. They were calculated from the formula explained in Vol. I, Section 192, viz.,

$$E = A - 10{\cdot}6N + 0{\cdot}22G$$

The wort solids were calculated from (sp. gr. − 1000) ÷ 4. Their apparent maltose content was taken as 65%. The permanently soluble nitrogen was calculated as 36% and 30% of the total nitrogen of the English and Californian malts respectively, formol nitrogen being assessed at 25% of the P.S.N. The results are expressed as grams per 100 ml. wort and may be converted to percentages on dry malt by multiplying by 10, under the assumption that the wort contained the extract of 10 grams dry malt per 100 ml. The figures given in succeeding Sections to illustrate the influence of varying mashing conditions on different grists are from actual analyses, many being taken from Horace Brown's[1] investigations on the nitrogen question in brewing. The concordance between the analytical and calculated figures supports the contention that the composition of wort is regularly related to that of the barley and that differences in wort composition can be referred to known changes in malting or mashing conditions. The influence of the former was dealt with in Chapter IX.

TABLE 164.—TYPICAL ANALYSES OF WORTS FROM ENGLISH AND CALIFORNIAN MALTS, CALCULATED FROM NITROGEN CONTENT AND THOUSAND-CORN WEIGHT

Nitrogen % on dry malt	Extract lb. per Qr. dry malt	Grams per 100 ml. extract wort				
		Total solids	Maltose	P.S.N.	Protein	Formol N
English malts						
1·3	102·4	7·62	4·95	0·048	0·300	0·012
1·5	100·3	7·44	4·84	0·054	0·338	0·013
1·7	98·2	7·30	4·75	0·061	0·381	0·015
1·9	96·1	7·15	4·65	0·068	0·425	0·017
Californian malts						
1·3	96·9	7·21	4·69	0·039	0·244	0·010
1·5	94·8	7·05	4·58	0·045	0·281	0·011
1·7	92·7	6·89	4·31	0·051	0·319	0·013
1·9	90·6	6·74	4·21	0·057	0·356	0·014

(435) Unmalted Grain and Wort Composition.

Employment of a proportion of unmalted cereals, in the form of flakes or grits, usually results in an increase of extract and some change in the carbohydrate composition of wort, indicated by a rather higher $[\alpha]_D$ under similar mashing conditions. Apart from this, their influence on wort composition is shown mainly by a reduction in the permanently soluble nitrogen and buffering power. The unmalted cereal is generally regarded as a source of extract yielding starch and nitrogen diluent. It is sufficiently accurate in practice to calculate the reduction in wort nitrogen from the percentage of malt replaced. Hopkins and Burns,[2] however, found that a rather greater quantity of nitrogen was extracted from malt and maize flakes than from a corresponding mixture of malt and pure starch. Their results, Table 165, suggest that the proteins of maize flakes are not completely resistant to the proteolytic enzymes of malt. The figures refer to mashes made with 150 grams of malt, and 100 grams of malt with 50 grams of maize flakes or starch in 360 ml. of water at 150° F., a stand of 2 hours being allowed before the total weight was made up to 650 grams.

TABLE 165.—WORTS FROM MALT AND MALT WITH MAIZE FLAKES OR STARCH (HOPKINS AND BURNS)

Grist	Wort p_H	Wort solids %	Total Nitrogen	
			grams per 100 ml.	% on wort solids
Malt	5·5	19·87	0·151	0·759
Malt and starch	5·5	20·22	0·100	0·493
Malt and maize	5·6	20·05	0·107	0·534

(436) Enzymic Activity of Malt and Wort Composition.

The inadequate information on the activity of the starch-converting enzymes of malt given by the Lintner and other analytical methods, particularly their failure to assess that due to insoluble enzymes (Vol. I, Sections 142 and 177), precludes any direct relation between the results of these determinations and the extent of starch conversion in the mash tun or apparent maltose content of the wort. This may be illustrated by analyses made a number of years ago by J. O'Sullivan.[3] He assessed the "starch-transforming power" of the malt by determining the apparent maltose in standard extract and cold water extract mashes and expressed it as a percentage of the starch content of the malt (Table 166).

There is also no relation between the extracts of malts of the same type and their diastatic activities when, as is normally the case, the conversion of starch to soluble products is complete.

Table 166.—Starch-Transforming Power of Malt (J. O'Sullivan)

Malt	Starch %	Transforming power	Diastatic activity Lintner °
1	4·122	88·0	58
2	4·515	82·0	58
3	4·629	91·2	49
4	4·890	89·7	31
5	4·925	82·3	26
6	4·384	60·2	13

The extract depends primarily on the starch content of the barley which, in similar barleys, varies approximately inversely with the nitrogen content, while the diastatic activity of malts usually increases with the nitrogen content, when malted under similar conditions. A higher extract is, consequently, generally associated with lower diastic activity in comparable malts. The influence of different mashing conditions on the carbohydrate composition of wort is, in addition, complicated by the existence in diastase of two enzymes with different properties, the α- and β-amylases. (Vol. I, Section 137.)

Analogous difficulties arise in any attempt to correlate the nitrogenous composition of wort with the proteolytic activity of malt. The extent of protein breakdown due to insoluble enzymes is illustrated by Kolbach and Simon's [4] analyses in Table 167. The figures under the heading "grains mash" represent the nitrogen fractions in the wort obtained by mashing the grains left after thoroughly extracting soluble enzymes with water at 68° F. This would also remove the ready formed soluble substances from the malt, including a quantity of permanently soluble nitrogen equivalent to about 18–20% of the total nitrogen of the malt. Allowing for this, it will be noted that a

Table 167.—Proteolysis by Insoluble Enzymes at Various Mashing Temperatures (Kolbach and Simon)

(N as % of total N of malt, 1·845%)

Temperature F.	Malt mash					Grains mash				
	68°	104°	122°	140°	158°	68°	104°	122°	140°	158°
Extract %	19·6	29·5	43·7	**79·3**	78·6	—	—	—	—	—
Soluble N	32·1	41·4	**44·6**	41·3	35·9	0·8	6·1	**12·4**	12·2	9·2
Coagulable N	9·5	9·6	8·3	5·1	2·5	0·1	0·4	0·4	0·5	0·6
Perm. soluble N ..	22·6	31·8	**36·3**	**36·2**	33·4	0·7	5·7	**12·0**	11·7	8·6
Formol N	10·2	13·6	**14·9**	14·2	12·6	0·4	2·3	**4·3**	3·6	2·2
Formol N as % of P.S.N.	**45·2**	42·8	41·1	39·4	37·8	—	**40**	36	31	26

much greater proportion of the P.S.N. and formol nitrogen of the wort from the malt mash is due to insoluble than to soluble enzymes. The decrease of coagulable nitrogen at higher temperatures is due mainly to coagulation during mashing.

These measurements of the products of insoluble enzymes account for the lack of any definite relation between ordinary determinations of enzymic activity, restricted to the soluble enzymes, and the composition of wort. The latter depends, apart from the composition of the malt, on the total activities of each of the enzymes concerned. The influence of the operative factors in mashing depends on the extent to which they activate or restrict these total activities or, on the other hand, prepare the insoluble constituents of the malt for their action. Nevertheless the determination of enzymic activities by available methods is some guide in practice. For example, it is advantageous to use malt with a high diastatic activity with unmalted cereals, and the difference between the diastatic activities of pale and dark malts is usually sufficiently marked to be correlated with differences in wort composition due to the total diastatic activity.

(437) Physical Condition and Preparation of the Grist.

The rate of enzymic reactions and the composition of the products are influenced by the physical or colloidal state of the substrate as well as by the activity of the enzymes. This is illustrated by the low results of diastatic activity determinations with imperfect preparations of soluble starch and by the need to prepare the starch of unmalted cereals by flaking or gelatinisation. The starch, protein and other constituents of malt can rarely be in a homogeneous state of preparation for conversion. Part may remain in the condition in which it existed in barley, and would require gelatinisation. Part is more or less modified and correspondingly amenable to enzymic attack after hydration, while part has been converted to soluble products during malting. A considerable loss of extract results when under-modified malts are coarsely ground with a single roller mill and mashed by infusion methods. Modern mills, however, deal adequately with hard ends and steely corns, without detriment to the porosity of the mash. The result is that there need be little loss of extract. There is also comparatively little variation in the carbohydrate composition of worts from under- and fully-modified malts, but considerable difference in their nitrogenous constituents. This is mainly due to the smaller percentage of preformed permanently soluble nitrogen in under-modified malts. The analyses in Table 168 refer to worts from laboratory mashes of two Plumage-Archer malts of closely similar nitrogen content.

TABLE 168.—COMPOSITION OF WORTS FROM WELL-MODIFIED AND UNDER-MODIFIED ENGLISH MALTS MASHED AT DIFFERENT TEMPERATURES

Temp. ° Fahr.	Extract dry malt lb. pr. Qr.	Wort solids gr. 100 ml. sp. gr. ÷ 4	Maltose		Perm. sol. Nitrogen		
			gr. 100 ml.	% on solids	gr. 100 ml.	% of solids	% of malt N
Well-modified malt, Nitrogen 1·45%							
120	59·8	4·45	2·80	62·9	0·056	1·26	38·6
140	100·3	7·46	**5·30**	**71·0**	**0·057**	0·76	**39·3**
150	**101·8**	**7·57**	4·92	65·0	0·056	0·74	37·9
160	100·6	7·48	4·10	54·8	0·049	0·65	33·8
Under-modified malt, Nitrogen 1·51%							
120	57·6	4·28	2·63	61·4	0·052	1·21	34·4
140	98·7	7·34	**5·23**	**71·2**	**0·053**	0·72	**35·1**
150	**100·0**	**7·44**	4·90	65·8	0·049	0·66	32·4
160	99·5	7·40	3·99	53·9	0·045	0·60	29·8

(438) Specific Heat and Heat of Hydration of Malt.

The moisture content of malt influences wort composition through its effects on the temperature of the mash. The heat evolved when malt and liquor are mixed is considerably greater with dry than with slack malt, as can be verified by noting the rise of temperature produced when malts of different moisture content are mixed with cold water. The inadvertent use of slack malt in an infusion mash may reduce the initial temperature by 3° or 4° F., as compared with dry malt mashed with liquor at the same temperature, with consequent change in wort composition. Variations in temperature due to this cause are of no consequence in a decoction mash with cold liquor, since little or no enzymic change occurs until the temperature is raised.

TABLE 169.—SPECIFIC HEAT AND SLAKING HEAT OF MALT (H. T. BROWN)

Malt moisture %	Specific heat	Slaking heat gram calories F.
0	0·38	28·0
1	0·38	24·7
2	0·39	21·5
3	0·40	18·2
4	0·40	15·8
5	0·41	13·5
6	0·41	11·5
7	0·42	10·0
8	0·42	8·5

Brown [5] found that perfectly dry malt evolved 28 gram calories F. per gram of malt on mixing with water at exactly the same temperature, that is to say, an amount of heat sufficient to raise 28 times its own weight of water through 1° F. or its own weight of water through 28°. This may be called the slaking heat or heat of hydration. The same malt with 4% of moisture only evolved 16 gram calories under similar conditions, while the heat evolution was only 8·5 calories with malt containing 8% of moisture. He gave the figures in Table 169 for the slaking heat and specific heat of malt with varying moisture content.

The striking heat necessary to obtain a predetermined initial temperature may be calculated from the above figures by means of the following formula, in which S = the specific heat of the malt, t° its temperature, R = the weight of water corresponding with unit weight of malt and T° its temperature. H is the slaking heat of malt and I° the initial temperature of the mash.

$$\frac{St^\circ + RT^\circ}{S + R} + \frac{\frac{1}{2}H}{S + R} = I^\circ$$

The first part of the equation gives the temperature that would result if there were no evolution of heat due to hydration. The second gives the number of degrees F. that must be added for this effect. With a slack malt, containing over 5% of moisture, the value of the latter becomes practically zero and the temperature of the mash is represented by the mixing heat only.

The temperature change produced by an underlet can be calculated from another equation in which S and R have the same significance as in the former and M° = the temperature of the mash at the time of underletting, Q the quantity of water used in the underlet and T° its temperature. F° = the final temperature of the mixture. Q is expressed in terms of unit weight of malt in the mash. In order to express it in barrels, it must be multiplied by $\frac{336}{352}$ or 0·95, and also by the number of quarters of malt in the mash, 336 and 352 being the relative weights of one quarter of malt and one barrel of water at mashing temperatures around 165° F.

$$\frac{(S + R) \times M^\circ + QT^\circ}{S + R + Q} = F^\circ$$

Variations in heat losses between liquor tank and mash tun restrict the general application of the first formula. It is preferable to substitute the full slaking heat for ½H, and make allowance for the average loss found by trial in any particular plant. Table 170 illustrates this by figures recalculated by Hopkins and Carter[6] from brewery observations by Brown and Morris.[7]

TABLE 170.—CALCULATED AND OBSERVED MASHING TEMPERATURES (HOPKINS AND CARTER)

No.	Malt		Mashing liquor		Mixing temp. °F.	Heat of hydration, °F.	Calculated initial °F.	Observed initial °F.	Difference
	Temp. °F.	Moisture %	Temp. °F.	Barrels per Qr.					
1	69	3·64	161·5	2·13	147·2	6·33	153·5	150·5	3·0
2	58	3·43	162·0	2·39	147·5	5·91	153·4	149·5	3·9
3	58	2·49	160·0	2·25	145·4	7·22	152·6	149·0	3·6
4	58	2·56	161·25	2·61	148·2	6·27	154·5	150·5	4·0
5	61	1·50	160·0	2·25	146·0	8·40	154·4	151·0	3·4
6	59	2·26	162·25	2·53	148·9	6·79	155·7	152·0	3·7
7	58	2·49	160·0	2·25	145·4	7·21	152·6	149·5	3·1
8	62	3·85	165·0	2·00	148·2	6·46	154·7	151·5	3·2

The temperature of the malt becomes of particular significance after retorrification, since that of the liquor must be reduced by 10° or more when mashing with warm grist. The effects on enzymic action do not appear to have been investigated, but many brewers claim that the check to their activity is reduced.

OPERATIVE FACTORS IN MASHING

(439) Influence of Temperature on Wort Composition.

It is possible to control the composition of wort, within limits that comprise a considerable range of fermentability and other properties, by taking advantage of the physical effects of hot liquor on the grist and by varying the temperature of the mash to activate or restrict enzymic changes. It was pointed out in Vol. I, Section 123, that enzymes were activated by increase of temperature to an optimum, at which the greatest quantity of reaction products was formed in a given time, and that this optimum was the resultant of a balance between activation and greater rapidity of destruction at higher temperatures. It may differ for each enzyme and varies with the time of reaction and with such conditions as the p_H value of the medium, the concentration and physical state of the substrate. The temperature optima for α-amylase and β-amylase in laboratory experiments are placed at 149° and 131° F. respectively, and the former is more thermostable than the latter. Since they are both concerned in starch conversion in the mash tun and their activities are affected differently by temperature and other conditions, there can be no fixed optimum for the greatest extract or most fermentable wort. Between 110° and 130° F., starch hydrolysis is due mainly to β-amylase, increasing in rapidity as the temperature approaches the optimum for that enzyme and the α-amylase is activated. Restriction of the saccharifying action of β-amylase above 135° or 140° is, to a certain extent, counterbalanced by continued increase of α-amylase activity and by the physical effect of hot liquor on the starch. β-amylase is progressively restricted until, at about 165° F., only α-amylase survives and is, in its turn, destroyed at about 175° or by exposure for a longer time to a lower temperature (see Section 467).

Mashing temperatures at which the greatest extract, most apparent maltose, permanently soluble nitrogen or formol nitrogen are obtained are higher than those usually found as optima in laboratory experiments with the enzymes concerned. They are not fixed, but vary within quite considerable ranges, according to the time during which they are allowed to act, the p_H value of the mash, its concentration and other factors. The optima are generally approximately as follows, under normal mashing conditions.

TABLE 171.—OPTIMUM MASHING TEMPERATURES

	F.	C.	R.
Highest extract yield	149°–155°	65°–68°	52°–55°
Most apparent maltose ..	140°–145°	60°–63°	48°–50°
Permanently soluble nitrogen ..	122°–140°	50°–60°	40°–48°
Most formol nitrogen	122°–131°	50°–55°	40°–44°

It is necessary to vary the temperature at different stages of mashing to take the greatest advantage of each of these optima, but one single temperature can be selected for an infusion mash of fully modified malt, with which it is unnecessary to stimulate proteolysis. The attack on transformable starch can be controlled to a certain extent by varying the mash tun temperatures, within the usual range of from 145°–155° F. Reduction of temperature within this range, with the same malt and liquor, increases the apparent maltose content of the wort, while higher temperatures have a restricting influence on starch hydrolysis. Variations of 1° or 2° F. have significant effects on the cupric reducing power and $[\alpha]_D$ of the wort and usually suffice to maintain the required regularity of wort composition, so far as it can be judged by these analytical determinations.

The figures in Table 172, from Brown's analyses, suggest an optimum for apparent maltose at about 144° F. Similar optima are obtained in laboratory mashes for both pale and dark malts, but the percentage of apparent maltose falls considerably with the more highly dried malts, with lower diastatic activity. Fermentability corresponds very closely with apparent maltose content, though the optimum temperature is generally found at about 148°–149° F. by determining the limiting attenuation of worts from mashes at different temperatures. The optimum for extract production is shown between 149° and 156° in Table 172, with very little reduction up to 163°. It is always several degrees higher than the apparent maltose optimum. The sugar/non-sugar ratios and $[\alpha]_D$ values show the increasing proportion of dextrins at higher temperatures. The rise of non-sugars and steep fall of $[\alpha]_D$ below 136° are, however, due to slow starch conversion at lower temperatures and active proteolysis. This is shown by the high percentage of nitrogen in the wort solids at 120° as compared with 140° in Table 173.

TABLE 172.—EFFECT OF MASHING TEMPERATURE ON THE CARBOHYDRATE COMPOSITION OF WORT, LABORATORY MASH (H. T. BROWN)

Temp. F.	Solids % g. 100 ml.	Apparent maltose		Sugar/non-sugar ratio	$[\alpha]_D$
		g. 100 ml.	% dry solids		
120°	5·68	3·42	60	100 : 66	88·9
128°	7·64	5·13	68	100 : 49	105·2
136°	9·88	6·75	68	100 : 46	116·8
144°	10·00	**6·77**	**68**	100 : 48	120·1
149°	**10·30**	6·43	62	100 : 60	124·1
156°	**10·30**	5·76	56	100 : 79	129·0
163°	10·20	4·89	48	100 : 109	130·8
170°	10·00	3·80	38	100 : 163	135·9

The influence of varying mashing temperatures on the total solid matter extracted and on the permanently soluble nitrogen of wort is illustrated in Table 173. The malts were mashed at the rate of 20 grams per 100 ml. and given 3 hours' stand. The difference in the nitrogen extracted from English and Californian malts is shown in this Table. The former had a total nitrogen content of 1·469% and a diastatic activity of 34·4° Lintner, the latter 1·548% and 22·4° Lintner. The optimum for P.S.N. was found at about 120° F. with the English malt and would probably have been somewhat higher for the Californian. The extract optima were at 150° and 160°, respectively. It is difficult to follow the total extraction of nitrogen, because a greater proportion is coagulated as the mashing temperature rises, so that the coagulable nitrogen in the wort is much reduced. The figures in Table 174 may be compared with these, and with those in Table 175.

TABLE 173.—EFFECT OF TEMPERATURE ON P.S.N. IN WORTS FROM ENGLISH AND CALIFORNIAN MALTS (H. T. BROWN)

Temp. Fahr.	English malt, N = 1·469%				Californian malt, N = 1·548%			
	grams per 100 ml.			P.S.N. % of N of malt	grams per 100 ml.			P.S.N. % of N of malt
	Solids*	Coag. N	P.S.N.		Solids*	Coag. N	P.S.N.	
60	3·49	0·019	0·065	23·0	3·27	0·014	0·063	21·5
100	4·89	0·020	0·085	30·5	3·93	0·010	0·075	25·8
110	5·87	0·018	0·103	37·1	—	—	—	—
120	7·73	0·017	**0·107**	**39·0**	5·67	0·006	**0·092**	**32·2**
130	11·72	0·015	0·102	37·9	—	—	—	—
140	13·18	0·008	0·099	37·4	—	—	—	—
150	**13·35**	0·007	0·092	34·8	12·59	0·004	0·087	31·8
160	13·30	0·007	0·083	31·3	**12·89**	0·001	0·081	29·6
180	12·75	0·007	0·060	22·7	—	—	—	—

* Solids calculated from specific gravity by divisor 4·0.

A second example of proteolysis in laboratory mashes of an English malt is given in Table 174 to emphasise the relatively large proportion of the permanently soluble nitrogen preformed in the malt and found by analysis of the extract at 32° F. The smaller quantities produced by proteolysis during mashing are given by the differences between analyses of the worts and cold water extract. Rather more or less than two-thirds of the P.S.N. of the wort from mashes at the optimum temperature is preformed in the malt, according to malting conditions. In this case the malt was mashed by the Institute of Brewing Standard method and the curve for permanently soluble nitrogen is almost flat between 120° and 150° F., the greatest quantity being found at 140°.

TABLE 174.—PROTEOLYSIS IN LABORATORY MASHES OF ENGLISH MALT NITROGEN CONTENT 1·45%

Mash temperature F.	32°	70°	120°	140°	150°	160°
P.S.N. as % of malt	0·36	0·41	0·56	**0·57**	0·56	0·49
,, % of malt N ..	24·8	28·3	38·6	**39·3**	38·6	33·8
Increase during mashing						
% of malt	—	0·05	0·20	**0·21**	0·20	0·13
% of malt N ..	—	3·5	13·6	**14·5**	13·6	9·0

Further information on proteolysis can be gained by formol titration of which the figures in Table 175 are an example.* They were derived from analyses by Kolbach and Buse [8] of worts from a pale lager malt, containing 1·65% of nitrogen on dry matter, mashed for 2 hours at the rate of 75 grams in 300 ml. of water at the temperatures stated and finally made up to 500 grams. The optimum for P.S.N. is again found at 140° F., with comparatively little change from 122°. The optimum for formol nitrogen is at 122°, but the curve representing its quantity between 104° and 140° is nearly flat. Similar optima are shown in Table 176. The most interesting point about the production of formol nitrogen is brought out by the figures for its percentage on P.S.N., that is by the amino-index of the substances represented by the permanently soluble nitrogen. This provides some evidence on the nature of the nitrogenous constituents of wort and on the debated question of peptidase action, which is dealt with in Section 470.

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* See footnote to Section 470.

TABLE 175.—PROTEOLYSIS IN LABORATORY MASHES OF PALE LAGER MALT AT DIFFERENT TEMPERATURES (KOLBACH AND BUSE)

Mash temperature F.	32°	104°	122°	140°	158°
P.S.N. % on dry malt ..	0·326	0·501	0·572	0·604	0·520
,, % malt N ..	19·7	30·4	34·7	**36·6**	31·5
Formol N % dry malt ..	0·135	0·201	**0·224**	0·208	0·183
,, % P.S.N. ..	41·4	40·1	39·2	34·4	35·2
Increase during mashing					
P.S.N. % dry malt ..	—	0·175	0·246	**0·278**	0·194
Formol N ,, ..	—	0·066	**0·089**	0·073	0·048
,, % P.S.N. ..	—	37·7	36·2	26·3	24·7

The influence of mashing temperatures on wort composition is illustrated in Fig. 112 by curves constructed from Brown's analyses of worts from the English malt, Tables 172 and 173. A curve representing the permanently soluble nitrogen as a percentage of the total nitrogen of the malt has the same form as that given for the nitrogenous constituents of the wort, which is much flatter than those for apparent maltose or extract and shows comparatively little variation between 110° and 140° F. This agrees with other experiments, though the greatest quantity of P.S.N. is found at 140° in some cases. A sharper inflection, with a maximum within the same range, is obtained if the P.S.N. produced in the mash is plotted instead of the total quantity in the wort, on account of the comparatively large proportion preformed in the malt. A curve for formol nitrogen, constructed from Kolbach and Buse's figures for its percentage on P.S.N., corresponds closely in form with the curve for P.S.N.

It will be noted that the permanently soluble nitrogen extracted from the malt under fixed conditions increases slowly but almost uniformly with temperatures between 60° and 100° F. The latter temperature coincides with what Brown called the "first critical point." A further rise of 10° results in a comparatively rapid increase in the P.S.N., as evidenced by the steeper gradient of the curve between 100° and 110° F. Beyond this point the curve, although still rising, begins to flatten considerably and attains its highest elevation at about 120° F., which marks the point at which the proteolytic enzymes are exerting their maximal effect on otherwise insoluble proteins of the malt. Between 120° and 140°, the P.S.N. shows a slight but appreciable decrease, indicating a region of temperature which is beginning to restrict

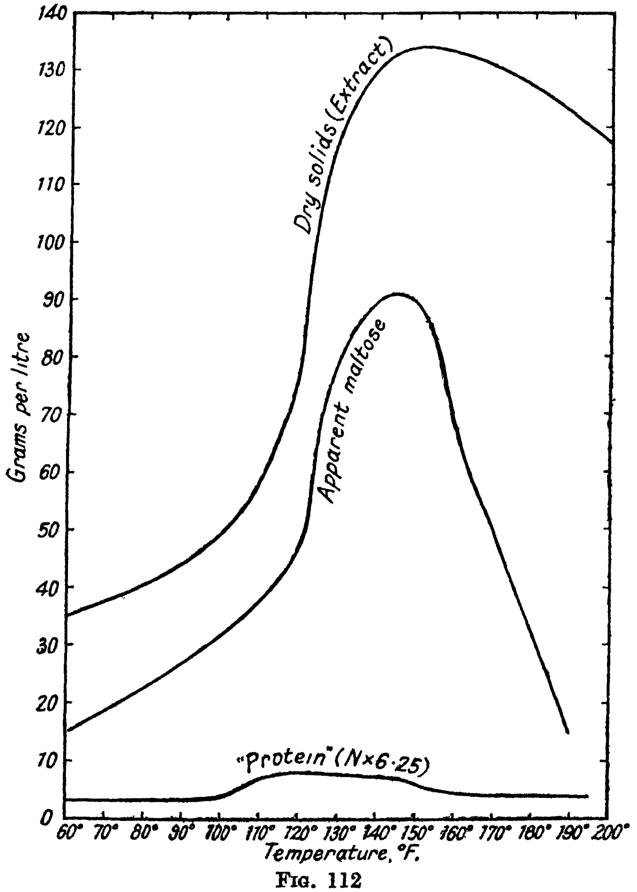

FIG. 112

INFLUENCE OF MASHING TEMPERATURE ON WORT COMPOSITION (THE CURVES SHOW GRAMS PER LITRE IN WORTS FROM AN ENGLISH MALT MASHED FOR 3 HOURS AT THE RATE OF 200 GRAMS PER LITRE) the action of the proteolytic enzymes. This effect becomes more marked above 140°, and at 150° another critical point is reached at which the descending curve becomes steeper. At about 180°, all proteolysis in the mash has ceased and the quantity of P.S.N. in the wort is about the same as in a cold water extract of the malt.

Though the quantity of nitrogen extracted at 60° and 180° F. may be the same, it by no means follows that the nitrogenous extractives are of the same nature. There is slow proteolysis of soluble nitrogenous substances at 60° F., which would not occur in extracts at 32°, and Brown pointed out that a larger proportion of the nitrogen extracted at 60° was available for yeast nutrition than in extracts at 180°. Brown determined the assimilable nitrogen in worts by two successive fermentations, the second being carried out with fresh yeast, after removal of alcohol from the residue of the first and replacement of fermented sugar. His differentiation of the "readily assimilable" nitrogen of the first fermentation and the "more difficultly assimilable" nitrogen of the second, gives some indication of a qualitative change in the nitrogenous substances. The differences, Table 176, are comparatively small and correspond more or less with determinations of formol nitrogen. They show a definite restriction in assimilable nitrogen or increase in the more difficultly assimilable fraction at mashing temperatures above 150° F.

TABLE 176.—INFLUENCE OF MASHING TEMPERATURE ON ASSIMILABLE NITROGEN (H. T. BROWN)

Mash temp. F.	Assimilable nitrogen % P.S.N.			Assimilable nitrogen % malt nitrogen		
	First fermentation	Second fermentation	Total	First fermentation	Second fermentation	Total
60°	**56·8**	4·56	61·3	13·0	1·0	14·0
100°	55·5	6·36	61·8	16·9	1·9	18·8
110°	53·5	9·3	**62·8**	**19·8**	3·4	23·2
120°	49·0	12·29	61·2	19·1	**4·8**	**23·9**
140°	46·6	12·53	59·1	17·4	4·6	22·0
150°	47·8	12·67	60·4	16·6	4·4	21·0
160°	42·5 ?	10·87	53·3	12·4	3·1	15·5
180°	43·8	**15·68**	59·4	9·9	3·5	13·4

(440) Protein Rest.

The points brought out in the preceding Section and Fig. 112 in regard to the maximum production of permanently soluble nitrogen at about 120° F. explain the influence of an initial mash stand at this temperature on wort composition. This is the "protein rest" or "peptonisation stand" of half an hour or an hour at 122° F. (50° C. or 40° R.) so often adopted in the decoction and American systems of mashing. It is of particular advantage with malts in which proteolysis has not been brought to such an advanced stage during malting as it is in most English malts. It produces further hydrolysis of otherwise insoluble nitrogenous malt constituents, as is shown by increase in the permanently soluble nitrogen of the wort, but it is questionable whether this is associated with simplification of the soluble nitrogenous substances (see Section 470). Reference to Table 175 shows, by the differences between the analyses at 32° and 104° or 122° F., that the increase in the quantity of formol nitrogen is proportional to that of the permanently soluble nitrogen, or that there may be a slight decrease in its percentage on the latter. This does not support the contention that the advantages of a digestive mash are always due to advanced proteolysis. It would appear that the composition of the permanently soluble nitrogen, in so far as it may be judged by its "amino-index" (Vol. I, Section 113) remains practically constant up to 122° and the same as that of the preformed permanently soluble nitrogenous constituents of the malt, as shown by extraction at 32° F. At higher temperatures there is apparently a change, the reduction in amino-index suggesting that the extracted nitrogenous substances become more complex.

Some other reason than proteolysis must, therefore, be sought in such cases for the improvement in stability and resistance to haze of the beer associated with a low temperature digestive mash. It may possibly be connected with the activation at 122° F. of enzymic breakdown of pectins or other of the hemicellulosic constituents of malt. Enzymes of the cytase group, that may be supposed to attack these substances, appear to be most active at temperatures slightly lower than the optimum for proteinase. (Section 128.)

(441) Action of Phytase.

Malt usually contains about 1% of P_2O_5, of which some 20% has been converted from its original organically combined forms to soluble potassium phosphates. Another 50% of the P_2O_5 may be taken into solution in an infusion mash by the action of phytase. This enzyme is most active at about 120° F. so that a protein rest may also be expected to increase the hydrolysis of organic phosphates to phosphoric acid, with production of potassium phosphates. This may be an important function of the digestive mash and a more probable explanation of the fall in p_H value that occurs, when the normal value is high, than development of acid-forming bacteria in the short time allowed (see Section 460). Analyses by Windisch and Reiser[9] showing phytase action are given in Table 177.

TABLE 177.—PHYTASE ACTION DURING PROTEIN DIGESTION MASH AT 122° F. (WINDISCH AND REISER)

Protein rest minutes	Total P_2O_5 in wort grm. per litre	Inorganic P_2O_5 as % of total P_2O_5
10	0·1630	77·2
20	0·1645	83·2
30	0·1660	88·0
60	0·1671	92·1

(442) Saccharification of the Mash.

Temperatures that are most favourable to proteolysis are considerably too low for the maximum production of maltose and still more so for the most rapid conversion of starch into products which will no longer give a colour with iodine. A mash is commonly stated to be " saccharified " immediately an iodine reaction is unobtainable with a sample taken from it. The term is misleading, since iodine ceases to indicate the presence of starch or dextrins before the maximum quantity of maltose is formed. " Saccharification " in this sense only marks that stage in the conversion at which starch or the higher dextrins have ceased to exist or until the colloidal state of the substrate is sufficiently changed to give no colour with iodine solution. " Saccharification time " is similarly loosely used to denote the period that elapses between the completion of mashing, in infusion systems, and the moment at which iodine ceases to give a colour reaction. In decoction systems, the time is measured from the moment at which the conversion temperature is reached. The optimum temperature for this stage of enzymic simplification, that is the mashing temperature with which it will be reached most rapidly, is much higher than the optimum for maltose production, as is shown by Kolbach's[10] figures in Table 178. These represent results obtained with a rather resistant malt, mashed at 45° C. (113° F.) by the Congress method and, after a rest of half an hour, raised to the temperature stated at the rate of 1° C. per minute. The times were measured from the moment at which this temperature was reached.

It will be noticed that iodine ceased to give a colour reaction before 172·4° F. was reached or that " saccharification " was attained during the 33 minutes required to raise the temperature from 113° to 172·4°. This stage of conversion would thus appear to be effected most rapidly at a temperature nearer to 172·4° than between 160° and 165° F., within which range the optimum was generally supposed to be. "Saccharification" is more rapid in dilute than in more concentrated mashes. Thus only 5 minutes was required with a mash giving a wort of 1056 sp. gr. while 10 and 15 minutes respectively were needed for mashes giving worts of 1092 and 1110.

TABLE 178.—RATE OF MASH SACCHARIFICATION (P. KOLBACH)

Temperature		Time minutes
C.	F.	
68°	154·4°	35
70°	158°	20
72°	161·6°	15
74°	165·2°	10
76°	168·8°	5
78°	172·4°	0

The difference between the temperatures required for most rapid "saccharification" and greatest maltose production is explained by the greater thermostability of α-amylase in comparison with β-amylase. The former is mainly responsible for breaking starch into dextrins that give no colour with iodine, and production of maltose by the latter or by both enzymes together is not given time for completion before the enzymes are destroyed at the higher temperature. As a result of this, wort from a mash made at a high temperature is less fermentable than that produced at a lower temperature, at which a longer time was required for "saccharification." Advantage is also taken of the rapid action of α-amylase at comparatively high temperatures by holding the mixture of raw grain and malt at about 160° F. for half an hour before boiling in the process of gelatinisation of a grits mash.

(443) Mashing Process.

Variations in wort composition result not only from changes in the initial temperature, but also from the manner in which the temperature is raised by underlet or decoction and the times at which the changes are made. With less well modified malts, it is not advisable to mash at high temperatures to hasten "saccharification." Better results are usually obtained by slow action at comparatively low temperatures, followed by an underlet.

Such initial temperatures as 145°–148° F., with a rise to 153° or 155° by underlet, are commonly adopted. It was mentioned in Section 404 that the time at which the underlet was given and its temperature had a material influence on the apparent maltose content of wort. Increasing the stand at an initial temperature lower than 150° increases the apparent maltose, while a shorter stand, followed by high underlet, decreases it. An example of the influence of a small variation in the mashing procedure on wort composition is given in Section 445 and Table 182. An increase of extract does not always result from the use of an underlet after a comparatively low initial. The greatest advantage is with under-modified malts. The single mash, with a higher initial, gives equally good results with fully modified malts.

Greater opportunities for controlled variation in wort composition are afforded by decoction systems than by infusion mashing, since greater or smaller proportions of the mash can be boiled and the stand at selected temperatures can be extended or shortened at will. The proportion of the mash boiled is not very different in a three-mash process, in which ⅓ is taken each time, or in a two-mash process, in which ½ and ⅓ are boiled, but considerable temperature differences result, with marked influence on the activity of enzymes and degree of conversion. Maltose formation and fermentability are considerably increased if the mash is given a stand of a quarter of an hour at between 140° and 149° F. during raising to the boiling point. The rapid mashing process with an initial of 144° also gives much maltose, because a large proportion of the mash stands at this favourable temperature for a considerable time, the quantity boiled being comparatively small. If the initial is reduced to 132°–136° to activate proteolysis, a less fermentable wort results, because maltose formation is slower at this temperature and more of the mash must be boiled to bring it up to 167° F. A more fermentable wort is usually produced in an infusion mash than in double or triple decoction systems, on account of the long stand at temperatures favourable to maltose production.

The analyses in Table 179, by Kolbach,[11] show, by comparisons between brewery and laboratory worts, that greater proteolysis occurs in the three-mash than in the two-mash system and that it can be intensified in the latter by introducing a protein rest or by acidification of the mash. (Section 449.) To what extent proteolysis should be carried with particular materials or activated by special mashing procedure must be judged by trial and comparison of the resulting beers. Measurement of the relation between the P.S.N. of the copper wort and laboratory wort from the same malt, expressed, according to Kolbach, as the "intensity value" of the process, provides a convenient method of comparison in such trials.

TABLE 179.—INFLUENCE OF MASHING PROCESS ON THE NITROGEN OF WORT, MEASURED BY P.S.N. OF FINISHED HOPPED WORT (P. KOLBACH)

Mashing process	N % of dry malt	P.S.N. as % of N of malt		Intensity value
		Copper wort	Congress wort	
Three-mash decoction	1·820	36·2	33·2	109
Two-mash „	1·656	35·6	37·6	95
„ biologically acidified	1·656	38·6	37·6	103
„ with peptonisation ..	1·730	34·9	33·1	106

(444) Mashing Rate.

The usual mashing rate in the infusion system is between 2 and 2½ barrels of liquor per quarter of malt. Greater concentration leads to somewhat increased proteolysis, but the extract may be reduced. Comparisons made between the permanently soluble nitrogen of the standard extract mash and another made with the same malt at a rate corresponding with that of a brewery mash, without any complications due to sparging or time factor, show an increase that varies with different malts, but may amount to about 10% of the P.S.N. of the laboratory mash, expressed as a percentage on the malt. Comparisons between the P.S.N. of Congress worts and copper worts in Table 179 show this as the maximum increase for the malts used. The analyses in Table 180 by Kolbach and Buse[8] represent worts obtained by mashing the same malt with water at 140° F. and at rates corresponding approximately with 1·1, 4·0, and 5·7 barrels per quarter, all being made up to 500 grams after a stand of 1 hour.

TABLE 180.—INFLUENCE OF MASHING RATE (KOLBACH AND BUSE)

Mash rate, weights malt : water	1 : 1	1 : 3·7	1 : 5·3
Extract of wort, % Plato ..	10·00	10·72	11·37
Perm. sol. N as % on malt ..	0·568	0·544	0·542
Formol N as % on malt ..	0·194	0·186	0·185

In some experiments on the influences of mashing temperature and mash concentration with a pale lager malt, containing

1·845% of nitrogen, mashed at the rate of 75 grams per 400 ml. of water (about 5 barrels per quarter), Kolbach and Simon[4] found the greatest production of P.S.N. at 131° F. and of formol nitrogen between 115° and 122°. By doubling the concentration of the mash, 8% more nitrogen was extracted, but the increase in P.S.N. was only 3%. These authors were inclined to ascribe the greater part of the increase of soluble nitrogen to greater solubility of more complex proteins in the salt solution provided by the mineral matter of the malt. The coagulable nitrogen was increased by 21% of its value in the dilute mash, but the formol nitrogen was reduced by 4%. The increase of permanently soluble nitrogen at higher concentrations may possibly be due to protection of the proteinase and surface effects.

(445) Duration of the Mash.

In an ordinary infusion mash, conversion is most rapid at the start and gradually slackens. A great proportion of it is effected during the first half-hour, but there is a small increase of extract and in the quantity of maltose and permanently soluble nitrogen after this, particularly with steely malts. The conversion is much slower when the initial is below 140° F. and it is only when the temperature is close to 150° that the full extract is obtained within 1½ to 2 hours. Analyses by Brown are given in Table 181 to exemplify this.

TABLE 181.—INFLUENCE OF DURATION OF THE MASH ON EXTRACT AND PERMANENTLY SOLUBLE NITROGEN (H. T. BROWN)

Temperature of Mash, F.	120°		150°	
Time of Stand	Extract % dry malt	P.S.N. % of N	Extract % dry malt	P.S.N. % of N
15 minutes	20·3	24·6	63·4	27·7
30 „	24·8	28·0	67·1	30·7
1 hour	28·2	32·2	69·4	32·9
1½ „	30·3	34·6	69·7	33·7
2 „	34·0	36·5	69·7	34·6
3 „	37·1	39·0	69·0	34·6

In view of the importance of the time factor in determining the most favourable period for the "stand on" of a brewery mash, Brown had a mash made with 52½ quarters of malt, at a rate of 2 barrels to the quarter, with special care to maintain the initial of 150° F. constant throughout by means of a steam-heated wort circulator. Samples drawn at intervals of a quarter of an hour gave the figures in Table 182. These show that the increase of extract was continuous up to 2 hours 20 minutes, but that the increase was comparatively small after 35 minutes. The $[\alpha]_D$ indicated practically no change in the carbohydrate constituents of the extract and the stand-on time had very little influence on its permanently soluble nitrogen content.

TABLE 182.—INFLUENCE OF "STAND ON" TIME IN A BREWERY MASH (H. T. BROWN)

Time from finish of mash	Extract lb.	$[\alpha]_D$	Permanently soluble N %	
			on dry extract	on dry malt
35 minutes	89·8	119·3	0·678	0·473
50 „	90·8	121·4	0·667	0·469
1 hour 5 „	91·0	121·1	0·664	0·469
1 „ 20 „	91·0	122·9	0·658	0·465
1 „ 35 „	91·6	121·3	0·675	0·479
1 „ 50 „	92·7	120·3	0·681	0·490
2 „ 5 „	93·4	119·6	0·691	0·500
2 „ 20 „	93·9	120·5	0·711	0·518
2 „ 35 „	93·9	120·6	0·711	0·518

By reducing the initial temperature to 146·5° F. and gradually raising that of the mash by means of the circulator to 153·7° in 1 hour 15 minutes, an increase of the permanently soluble nitrogen to 0·749% on the dry extract, or 8%, was obtained after 2 hours' stand. This brings out clearly the influence of comparatively small variations in mashing temperature on the permanently soluble nitrogen, at about 150° F., which is a critical point as regards the action of proteolytic enzymes. The $[\alpha]_D$ was also reduced to 115·7°, while the extract was increased to 94·6 lb.

The temperature at which the greatest quantity of the products of an enzymic reaction is produced in a given time must vary with the time, because it depends on the balance between activation and destruction of the enzyme. The quantity of product after a certain interval at a given temperature may be greater than after the same time at a lower temperature, but less after longer exposure to the higher temperature than at the lower, if destruction becomes more significant than activation. In other words, the optimum temperature for any enzyme falls with increasing time of action. Kolbach and Buse, for example, found that the optimum for production of permanently soluble nitrogen in their experiments at about p_H 6·0 was at 140° F. for half-hour stand, at 136·4° for one hour, at 134·6° for 2 hours and fell to 129·2° with a stand of 4 hours.

(446) Summary.

The composition of wort depends primarily on the composition of the grist and on the extent of enzyme action on its insoluble and soluble constituents during mashing. The influence of the operative factors in mashing depends on the degree to which they activate or restrict the activities of the various enzymes or prepare the insoluble constituents of the grist for their attack. Among the factors for preparation of the substrates for enzymic action are modification during malting and gelatinisation of raw grain. Then the manner in which the malt is ground and the boiling to which it is subjected in decoction mashing systems both influence the readiness with which starch, proteins and other malt constituents are broken down by the enzymes. The operative factors, by which the brewer is enabled to control the degree of enzymic breakdown, are temperature, acidity, mashing process, mash rate and duration of the mash. The acidity of the mash depends very largely on the composition of the liquor which, with methods for adjusting the p_H value, is dealt with in the next Chapter.

TABLE 183.—TYPICAL VALUES FOR P.S.N. OF BARLEY, MALT AND WORT (% OF TOTAL NITROGEN OF BARLEY)

	Two-rowed English	Six-rowed Californian	Moravian Lager malt
Barley, cold water extract ..	10	8	9
Malt, cold water extract ..	20	15	19
Laboratory wort	36	30	34
Brewery wort	40	33	36
(Congress and decoction mash for lager malt.)			

The effects of these factors on wort composition are most readily detected by determinations of extract, reducing power, and nitrogen content of the wort. Each of these is raised to a maximum under conditions that approximate to the experimentally ascertained optima of the enzymes concerned, though the temperature optima are definitely higher in the mash than in laboratory experiments. Correlation of the optima for extract and reducing power or for proteolysis with those ascribed to individual enzymes is complicated by the co-operation of more than one enzyme in each process. The figures in Table 183 represent approximately the extent of proteolysis during malting and mashing with normal malts. The values given for the worts can be raised or lowered by changes in the operative factors. Starch conversion during mashing provides a much greater proportion of the total extract and apparent maltose of the wort, but the influence of the various factors can similarly be shown by variations in the specific gravity and reducing power of the worts.

It is particularly to be noted that definite optima for the influences of temperature and other mashing conditions on wort composition cannot be given, unless all other factors are specified. The time of action is most significant in this respect. It may, for example, be found that maltose production is greatest at 145° F. in certain circumstances, but the optimum would be at a higher temperature, if the duration of the mash were shortened, and at a lower temperature, if conversion were allowed to proceed for a longer period. Both temperature and time are required to destroy enzymes. Higher temperatures increase their activity for a period, as well as accelerate their destruction. The principle of balance between activation and destruction must always be remembered in connection with enzyme action. It applies to all the factors that influence wort composition through their effects on enzymes. Another example of the influence of one factor on another is given by Kolbach and Haase's[12] results in Table 184. These show how the optimum temperature for extract and maltose production is influenced by the reaction of the mash, other conditions being constant.

TABLE 184.—RELATION BETWEEN p_H AND TEMPERATURE OPTIMA IN A MALT MASH (KOLBACH AND HAASE)

Temperature optima, °F., at varying p_H

p_H	For Extract	For Maltose
6·6	131°–140°	131°–140°
6·0	140°–149°	131°–140°
5·1	131°–140°	122°–131°
4·5	122°–131°	113°–122°
4·2	113°–122°	95°–104°

p_H optima at varying temperatures, °F.

Temperature °F.	For Extract	For Maltose
68°–104°	4·0–4·5	4·0–4·5
122°	4·9–5·3	4·4–5·0
140°–158°	—	5·7–6·2
140°	5·1–5·5	—
149°	5·5–5·9	—

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CHAPTER XXVI

REACTION OF THE MASH AND COMPOSITION OF SPARGINGS

MASH AND WORT REACTION

(447) Influence of the Liquor on the Reaction of the Mash.

It was pointed out in Vol. I, Sections 333 to 338, that the mineral constituents of natural and treated brewing liquors have important effects on the composition of wort through their influence on the reaction of the mash. The explanation, based mainly on interactions between ions of the salts in the liquor and phosphates derived from the malt, was illustrated by equations suggested by Windisch and Goldacker,[1] which have been confirmed and extended by Hopkins and Amphlett [2] since the publication of Vol. I. The acid-reducing effects of carbonates are only approximately expressed by

$$4KH_2PO_4 + 3CaCO_3 = Ca_3(PO_4)_2 + 2K_2HPO_4 + 3H_2O + 3CO_2$$

and the increased acidity produced by gypsum by

$$4K_2HPO_4 + 3CaSO_4 = Ca_3(PO_4)_2 + 2KH_2PO_4 + 3K_2SO_4$$

Carbonates do not exert their full influence in the mash in the presence of free CO_2. Unless removed by boiling, the free CO_2 in natural waters or in acid-treated liquors causes the p_H value of the wort to be rather lower than would be expected from the quantity of carbonate present. Heron [3] noted a case in which the quantity of free CO_2 in the original water was so large that it was impossible to obtain any regularity in the p_H value of the wort by acid treatment of the liquor. The full effect of carbonates on the p_H of worts is only exercised when the CO_2 is driven off by boiling in the copper. The precipitation of $Ca_3(PO_4)_2$ is also only partial in the mash. These points, which are of importance in stabilising the p_H value of worts, are dealt with in Section 448.

Tricalcic phosphate appears to be the only calcium salt of phosphoric acid precipitated, but Hopkins and Amphlett found that a good deal of the acidifying influence of gypsum in the mash tun was due to precipitation of compounds of calcium and organic phosphates. Conversion of the latter to inorganic phosphates by phytase during mashing is comparatively slow, unless the activity of the phosphatases is high, as it apparently is with highly diastatic malts. It is, however, an essential preliminary to the precipitation of $Ca_3(PO_4)_2$, which occurs mainly in the copper. Precipitation of tricalcic phosphate is also hindered by the presence of other salts, whether derived from the liquor or the malt. This applies particularly to magnesium salts, which may be derived from the malt in quantity equivalent to 60 parts per 100,000 of $MgSO_4$. Since the basic effect of carbonates is counteracted by the acidifying action of calcium ions, the total effect on wort p_H of a liquor containing considerable proportions of calcium in the form of sulphates or chlorides as well as carbonates may resemble that of a soft liquor, free from carbonates. Magnesium sulphate and chloride also oppose the acidifying influence of calcium by increasing the solubility of $Ca_3(PO_4)_2$. This effect is, however, offset by combination of magnesium with proteins. Sodium and potassium salts similarly hinder the precipitation of $Ca_3(PO_4)_2$, but do not combine with proteins. Additions of magnesium sulphate to the mashing liquor have little, if any, influence on the p_H of the mash or on the phosphate content of the wort.

TABLE 185.—INFLUENCE OF CALCIUM SALTS IN MASHING LIQUOR ON WORT COMPOSITION (HOPKINS AND AMPHLETT)

	1	2	3	4	5	6
Composition of liquor						
$CaSO_4$ pts. per 100,000 ..	0·0	27·2	81·6	40·8	54·4	81·6
$CaCO_3$,, ,, ..	0·0	6·9	10·3	0·0	1·7	3·45
As milli-equivalents per litre						
$CaSO_4$	0·0	4·0	12·0	6·0	8·0	12·0
$CaCO_3$	0·0	1·38	2·06	0·0	0·34	0·69
Composition of wort						
p_H before boiling	5·53	5·52	5·48	5·42	5·38	5·32
p_H after boiling 2 hours ..	5·35	5·35	5·21	5·23	5·15	5·12
Permanently soluble nitrogen, mgms. per 100 ml. ..	109	110	111	111	—	116
Formol nitrogen, mgms. per 100 ml.	23	23	22	22	22	22
Inorganic phosphate as P_2O_5 mgms. per 100 ml. ..	78·0	68·0	68·0	73·5	73·0	73·5
Inorganic phosphate as P_2O_5 milli-equivalents per litre	32·9	28·6	28·6	30·96	30·75	30·96
Calcium (Ca), mgms. per 100 ml.	5·52	6·62	17·02	9·32	11·70	16·78
Calcium, milli-equivalents per litre	2·76	3·31	8·51	4·66	5·85	8·39

Hopkins and Amphlett concluded that the salts have no influence on the permanently soluble nitrogen, formol nitrogen or maltose content of wort, other than that due to their effect on the p_H value, which causes an increase in the P.S.N. The precipitation of phosphate caused by a very hard gypseous liquor might, however, be sufficient to deprive the yeast of an adequate supply of an essential nutrient. The loss of inorganic phosphate with different liquors is shown in the personally communicated Table 185 by the difference between the quantity found, and that in the distilled water wort. The loss due to precipitation is, however, counterbalanced to some extent by increased production of inorganic phosphate in the mash, through activation of phytase as the reaction is brought more closely to the optimum of phytase at p_H 5·2. The resulting loss of inorganic phosphate is relatively small, compared with that precipitated by calcium in organic combination (Table 186), with which most of the calcium added in the mash liquor is also lost. The calcium in the distilled water wort was all derived from the malt.

Table 186.—Phosphates and Calcium in Worts (Hopkins and Amphlett) (phosphates expressed as mgms. P_2O_5 per 100 ml., calcium as mgms. Ca per 100 ml., *i.e.*, parts per 100,000)

Time (minutes)	15	30	60	90	120
Mash A (distilled water)—					
p_H	5·65	5·39	5·43	5·44	5·43
Phosphate — Inorganic	43·3	56·1	69·5	75·2	78·0
Phosphate — Organic	32·0	43·3	49·7	51·8	54·0
Phosphate — Total	75·3	99·4	119·2	127·0	132·0
Calcium (total)	7·1	4·0	3·6	—	4·1
Mash B ($CaSO_4 + CaCO_3 = 68·6 + 23·0$ pts. per 100,000)					
p_H	5·64	5·43	5·38	5·37	5·37
Phosphate — Inorganic	30·6	51·2	61·8	66·0	66·7
Phosphate — Organic	18·4	26·2	24·8	20·6	24·8
Phosphate — Total	49·0	77·4	86·6	86·6	91·5
Calcium (total)	19·1	15·5	12·5	10·7	11·3
Mash C ($CaCO_3 = 31$ pts. per 100,000)					
p_H	5·91	5·84	5·76	5·68	5·64
Phosphate — Inorganic	37·8	45·7	54·5	66·7	71·0
Phosphate — Organic	21·3	21·7	28·5	36·3	32·0
Phosphate — Total	59·1	67·4	83·0	103·0	103·0
Calcium (total)	7·5	6·8	5·2	5·3	4·5

The influence in brewing of the loss of organic phosphate caused by normal quantities of calcium sulphate or carbonate in the liquor is not yet known. It may, however, be recalled that phytin, which is the principal organic compound of phosphorus in malt, is a compound of phosphoric acid and inositol (Section 134) and the latter is a constituent of "bios." The progressive conversion of organic phosphate to inorganic by phytase during mashing is indicated in Table 186. The total quantity of phosphate lost is approximately equivalent to the loss of calcium. Since this loss is gradual, though most rapid during the first 15 minutes of mashing, the final p_H of the mash is attained more slowly in the presence of calcium salts.

(448) Reaction of the Mash.

The actual hydrogen ion concentration of the liquid part of a mash is rarely determined at the high temperatures existing in the tun. In published experimental work and in brewery practice it is usual to determine the p_H value of filtered wort at 20° C. and assume that this represents the reaction of the mash at a higher temperature. This is not actually the case, since there is greater dissociation of electrolytes at higher temperatures, resulting in a reduction of p_H value. For example, the p_H value corresponding with neutrality is 7·47 at 0° C., 7·07 at 20° C., but only 6·12 at 100° C. The p_H value of a mash at 150° F. may be about 0·3 lower than that of the wort, determined at the normal temperature. This, in part at least, explains the higher optimum p_H values attributed to enzyme activities in the mash tun compared with those found in laboratory experiments at lower temperatures and variations in the optima that apparently correspond with different mashing temperatures. Thus Windisch, Kolbach and Schild [4] concluded from determinations of the p_H values of the worts at 20° C. that the optima for production of permanently soluble nitrogen at different temperatures were those shown in Table 187, whereas the proteinase responsible for it would actually have been functioning at each temperature in a medium in which the hydrogen ion

TABLE 187.—p_H OPTIMA FOR PRODUCTION OF P.S.N. IN THE MASH AT DIFFERENT TEMPERATURES (WINDISCH *et al.*)

Mash temperature		p_H optima (determined in wort)
C.	F.	
30°–50°	86°–122°	4·5
60°	140°	4·7
65°	149°	4·8
70°	158°	5·1

concentration would be indicated by a p_H value below 4·5. This should be borne in mind in interpreting the p_H values given in the following pages. They were all determined in the wort at 20° C., no corresponding figures for actual mash reactions being available.

The reaction or "acidity" of wort is determined by the existence in solution of numerous buffer systems, including phosphates, amino-acids, carbonates, weak organic acids and their salts, etc. A typical electrometric titration curve for first length brewery wort by Hagues[5] indicates buffering through the entire p_H range, with only a few points where it is accentuated. It is impossible to draw very definite conclusions as to the buffering substances from such a composite curve, but there is no doubt that the phosphates contribute substantially above about p_H 5·5 and amino-acids and other products of proteolysis below this.

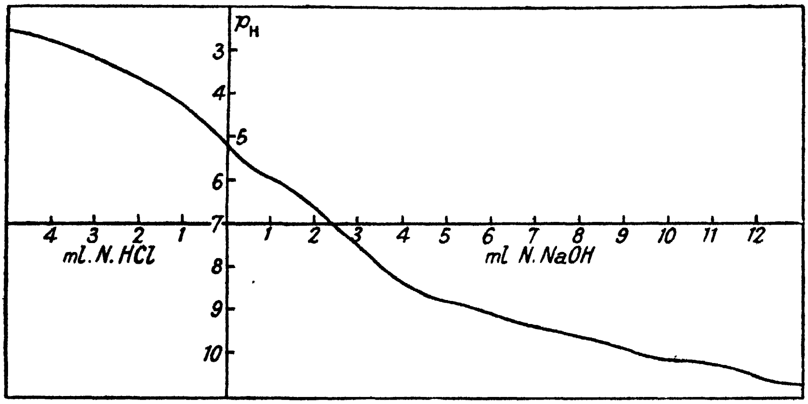

Fig. 113

ELECTROMETRIC TITRATION CURVE FOR FIRST LENGTH BREWERY WORT. ML. NORMAL ACID OR ALKALI PER 100 ML. WORT (G. HAGUES)

The reaction of a malt mash is always slightly acid, as measured by determination of the hydrogen ion concentration of the wort. That of the mash immediately after mixing the malt and liquor depends on the "acidity" of the malt and alkalinity of the liquor. The former is largely due to the liberation of phosphates during malting and only to a very small extent to the formation of lactic acid in ordinary malts. The alkalinity of liquors is due to their carbonate content. The wort from a mash of an English malt in distilled water usually has a p_H value of between 5·5 and 6·0. Dark malts generally give a lower p_H value than pale malts, and lager malts a higher value than English malts. The p_H value is not stationary during mashing, but gradually becomes more acid, largely through the liberation of inorganic phosphates by phytase and the action of proteolytic enzymes. The products contribute to the buffers of wort, as do such opposing systems as are formed by carbonates, and the whole is modified by salt reactions until the equilibrium indicated by the p_H value of the wort is reached.

(449) Reaction of the Mash and Wort Composition.

Although the hydrogen ion concentration of the hot mash is greater than that of the cold wort, which is determined for control purposes, its reaction is usually insufficiently acid to allow the proteolytic and some other enzymes to exert their greatest activity. This is illustrated by the curves in Fig. 114, which show the influence of variations in the p_H value, as determined in the wort, on the extract, cupric reducing power, total nitrogen and formol nitrogen of the wort, obtained by mashing an English malt for 2 hours at 150° F. (Hopkins [6]). The optimum p_H values under the experimental conditions employed were

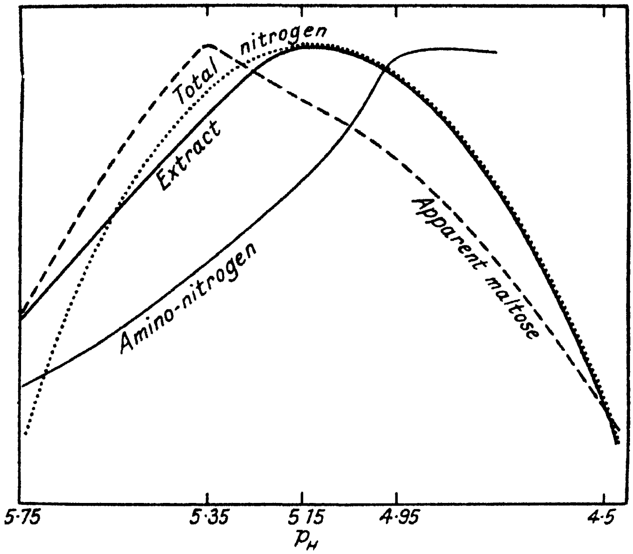

Fig. 114

INFLUENCE OF MASH REACTION ON WORT COMPOSITION (R. H. HOPKINS)

For extract	..	p_H 5·15
apparent maltose		5·35
total nitrogen		5·15
formol nitrogen		4·5–5·0

A third of the increased extract obtained at the optimum reaction was due to nitrogenous substances, and it will be observed that the optimum for formol nitrogen was much lower than those of malt peptidases, given as p_H 7·8 and 8·6 in Section 131. It may be concluded that the increase of formol nitrogen, resulting from a decrease in the p_H value of a mash at 150° F., is due to the action of proteinase (Section 470). A good break was obtained at as high a p_H value as 5·4. The optimum for apparent maltose or reducing power was considerably higher than that for proteolysis. The optimum reaction for the production of inorganic phosphates from organic compounds by phytase is at about p_H 5·2.

The effects on wort composition of variations in the p_H value of the mash may be studied from the results of laboratory experiments with different malts and mashing conditions, of which examples are given in Tables 188 to 191. The first set of analyses, by Kelly,[7] are of worts from an English-made Californian malt, of which 150 grams were mashed with 500 ml. of water treated with acid and alkali to adjust the p_H values. The maximum quantity of sulphuric acid to produce the lowest wort p_H was added in each case to keep the SO_4 ion concentration constant. Requisite additions of caustic soda were made to give the higher p_H values. The mashing temperature was 149° F. and the stand 2 hours.

TABLE 188.—INFLUENCE OF THE p_H VALUE OF A MASH AT 149° F. ON WORT COMPOSITION, CALIFORNIAN MALT (H. E. KELLY)
(NITROGEN IN MGMS. PER 100 ML. WORT)

p_H of wort	4·37	4·89	5·17	5·41	5·78	6·13
Perm. sol. N	130·7	**141·9**	137·2	131·6	120·4	106·4
Formol N	25·5	29·6	**31·3**	28·0	22·2	18·9
Formol N as % P.S.N.	19·5	20·8	**22·8**	21·3	18·4	17·7
Specific gravity ..	1065·8	1072·9	**1074·3**	**1074·4**	1073·7	1073·3
dA/dp_H 5–4	**1·06**	1·04	1·00	0·89	0·90	0·81
dB/dp_H 5-6·5	0·71	**0·82**	**0·81**	**0·83**	0·68	0·64

The figures for the buffering power of the wort are expressed in different ways in this and the next Table. Those in Table 188 are Van Slyke buffer coefficients and represent the number of ml. of 0·1N acid or alkali required to shift the p_H value of 10 ml. of wort by one unit, within the p_H ranges given. They are expressed as dA/dp_H or dB/dp_H, according as they refer to titrations with acid or base. The figures in Table 189 represent the number of ml. of 0·1N acid or alkali required to change the reaction of 100 ml. of the wort through the p_H range stated. Higher figures in either case represent greater buffering power. The most important buffers between p_H 7·07 and 5·67 or above

p_H 5·0 are the phosphates, and the figures are interesting as an indication of the production of inorganic phosphates in the mash by phytase or of the phosphates available for yeast food. Phosphates would still act as buffers below p_H 5·67, but the most important buffering in the range p_H 5·67–4·27 or below p_H 5·0 is due to proteolytic degradation products. Greater buffering in the lower ranges signifies increased resistance of the wort to development of a safe acidity during fermentation, but may mean increased assimilability of the nitrogen by yeast. About three-quarters of the buffers in the worts would have been present in the malts and soluble in cold water. The number of ml. of 0·1N alkali required to bring 100 ml. of the wort from its original p_H value to neutrality, p_H 7·07, is referred to by Continental workers as its "titration acidity" and the number of ml. of 0·1N acid required to bring them to p_H 4·27 as its "titration alkalinity." It is of interest to note that the buffering power produced between p_H 5·67 and 4·27 when the mash is acidified by lactic acid is from 5 to 10% greater than when a mineral acid is used. This is due to the presence of lactates and is a significant point in adjustment of the mash reaction in practice.

TABLE 189.—INFLUENCE OF THE p_H VALUE OF THE MASH ON CARBOHYDRATE BREAKDOWN AND BUFFERING (WINDISCH *et al.*)

(75 GR. PALE LAGER MALT IN 300 ML. WATER WITH NaOH OR HCl. TEMP. 158° F. AFTER HALF-HOUR PROTEIN REST AT 113° F. MADE UP TO 500 GR.)

p_H of wort	6·35	6·14	**5·98**	5·73	5·54	5·32	5·09	4·87
Extract % Plato ..	11·64	11·72	11·80	11·86	11·95	12·02	**12·08**	12·07
Maltose, gr. 100 ml. ..	8·60	8·86	9·01	9·27	9·29	**9·44**	9·36	9·16
„ % wort solids ..	70·6	72·2	72·9	74·6	74·2	**74·9**	73·9	72·3
Apparent attenuation %	77·1	77·9	78·5	**79·2**	**79·2**	78·3	74·9	68·3
Saccharification time ..	40′	20′	20′	**15′**	**15′**	**15′**	20′	20′
p_H of boiled wort ..	6·04	5·88	5·77	5·54	5·80	5·19	5·02	4·81
p_H of beer	4·69	4·73	4·64	4·47	4·40	4·37	4·41	4·28
Fall of p_H by fermentation	**1·35**	1·15	1·13	1·07	0·98	0·82	0·61	0·53
Buffers, 100 ml.								
p_H 7·07–5·67	8·44	9·55	10·67	11·15	12·31	13·17	**13·95**	13·70
p_H 5·67–4·27	6·66	6·23	7·22	7·85	8·54	9·14	9·04	**9·35**

Table 189 is from a comprehensive study of the influence of the p_H value of the mash by Windisch, Kolbach and Banholzer.[8] In this case 75 grams of pale lager malt were mashed with 300 ml. of water at 113° F. and, after a stand of half an hour for proteolysis, raised to 158° F. and maintained at that temperature for an hour, the weight of the mash being finally made up to 500 grams. The p_H value of the untreated wort was 5·98. The other values were obtained by 10 and 20 ml. of 0·1N NaOH or 10, 20, 30, 40 and 50 ml. 0·1N HCl. The different optima for total solids, reducing power or apparent maltose and fermentability should be noted. Total solids are given as grams per 100 grams wort or extract % Plato. The other figures are in grams per ml. The p_H values of the beer show the influence of the buffers in the boiled wort on the fall of p_H during fermentation.

The analyses in Table 190 (Kolbach and Buse[9]) show the joint influence of temperature and p_H on proteolysis during mashing; 75 gr. of pale lager malt was mashed with 300 ml. of a buffer solution containing sodium lactate, caustic soda and lactic acid in varying quantities for one hour at the temperatures given and finally made up to 500 grams. The extract at 32° F. represents preformed soluble malt constituents. The formol nitrogen produced in the mash, that is the difference between the quantities extracted at higher temperatures and at 32° F., is given as a percentage of P.S.N. produced, which was ascertained in the same way.*

TABLE 190.—INFLUENCE OF TEMPERATURE AND p_H ON PROTEOLYSIS (KOLBACH AND BUSE)

(NITROGEN AS MGM. PER 100 GRAMS DRY MALT)

Temp. F.	Analysis of worts						Produced in mash				
	p_H	6·5	6·0	5·5	5·0	4·6	6·5	6·0	5·5	5·0	4·6
32°	Perm. sol. N	331	327	334	**342**	**341**	—	—	—	—	—
	Formol N	127	127	129	132	**135**	—	—	—	—	—
	„ % P.S.N.	37	39	39	39	40	—	—	—	—	—
104°	Perm. sol. N	428	478	554	620	**645**	97	151	220	278	**304**
	Formol N	168	183	200	216	**224**	41	56	71	84	**89**
	„ % P.S.N.	**39**	38	36	35	35	**42**	37	32	30	29
122°	Perm. sol. N	445	530	632	712	**772**	114	203	298	370	**431**
	Formol N	157	187	219	242	**260**	30	60	90	110	**125**
	„ % P.S.N.	35	35	35	34	34	26	30	30	30	29
140°	Perm. sol. N	471	547	657	**728**	**727**	140	220	323	**386**	**386**
	Formol N	158	182	214	235	**242**	31	55	85	103	**107**
	„ % P.S.N.	34	33	33	32	33	22	25	26	27	27

The figures in Table 191 refer to the same mashes and worts as those of which the analyses were given in Table 189. They show the influence of variation in the p_H value of the mash, when a protein rest of half an hour at 113° F. was given. The experiments were also intended to find out whether any influence on the nature of the conversion products, such as might have caused a variation in the quantity assimilated by yeast during fermentation, could be detected. The smaller percentage of the P.S.N. assimilated by bottom in comparison with top fermentation yeasts (Table 176) will be noted.

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* See footnote to Section 470 for formol nitrogen in this and following Tables.

TABLE 191.—INFLUENCE OF THE p_H VALUE OF THE MASH ON PROTEOLYSIS AND NITROGEN ASSIMILATION BY YEAST (WINDISCH *et al.*)
(SAME WORTS AS TABLE 189—MASH INCLUDED PROTEIN REST)

p_H of wort	6 35	6·14	**5·98**	5·73	5·54	5·32	5·09	4·87
P.S.N. mgm. 100 ml. wort	88·3	92·0	97·9	107·3	116·3	124·6	131·9	**142·1**
„ 100 ml. beer	62·1	67·5	73·3	76·0	84·4	90·3	105·8	107·3
„ assimilated	26·2	24·5	24·6	31·3	31·9	**34·3**	26·1	**34·8**
„ % assimilated	29·7	26·6	25·1	29·2	27·4	27·5	20·0	24·5
Formol N % of P.S.N. wort	35·0	36·2	34·9	33·9	33·0	34·2	34·2	34·2
„ % of P.S.N. beer	29·5	27·7	27·0	26·3	24·6	26·7	27·8	29·8
„ mg. 100 ml. wort	30·9	33·3	34·2	36·3	38·4	42·6	45·1	**48·6**
„ mg. 100 ml. beer	18·3	18·7	19·9	20·0	20·8	24·2	29·4	**31·9**
„ assimilated ..	12·6	14·6	14·3	16·5	17·5	**18·4**	15·7	16·7
„ % assimilated	40·7	43·8	41·8	40·0	45·8	43·2	34·8	34·2

The most notable points brought out by the figures in these Tables are :—

(1) That the p_H value of the mash influences both the carbohydrate and nitrogenous composition of the wort.

(2) The enzymes are activated at all temperatures by increase of acidity resulting in a wort with p_H value slightly above or below 5·0.

(3) The proteolytic enzymes are activated to a much greater degree than the amylolytic by increasing acidity. The increase of P.S.N. and formol nitrogen at lower p_H values is much greater than that of extract, maltose or fermentability.

(4) The optimum for P.S.N. and formol nitrogen under different conditions varies from about p_H 5·15 to p_H values rather below 5.

(5) The p_H optima, as determined in the wort, appear to rise with increasing temperature. Kelly found that the optimum for permanently soluble nitrogen and formol nitrogen was at about p_H 5·0 when the mash was made at 149°–158° F., but below this at lower temperatures. His results are summarised in Table 192.

TABLE 192.—p_H OPTIMA AT DIFFERENT TEMPERATURES (H. E. KELLY)

Temperature F.	P.S.N. optimum	Formol N optimum
104°–122°	4·4–4·6	4·4–5·0
131°–149°	4·6–5·0	4·4–5·2
149°–158°	5·0–5·2	4·6–5·2

(6) The increase of formol nitrogen is almost parallel with that of P.S.N. under a wide range of conditions, suggesting that only one enzyme, proteinase, is active and that the increase of nitrogen is quantitative and not qualitative.

(7) This appears to be supported by the lack of any definite indication in Table 189 of variation in the quantity of P.S.N. or of formol nitrogen assimilated, other than might be ascribed to activation of yeast growth at p_H values somewhat above 5, corresponding roughly with the optimum for apparent attenuation in Section 438.

(8) The formol nitrogen produced in the mash at p_H 6·5 and 104° F., Table 188, does, however, form a distinctly higher percentage of the P.S.N. than under other conditions. This suggests that peptidase is active in some malts and that the nitrogen increase is qualitative as well as quantitative. The greater amino-index in such circumstances corresponds with the low thermostability and high p_H optima of the peptidases, which would probably be inactive in many malts or, if present, be inactivated in mashes at higher temperatures and greater acidity.

(9) There appears to be a fall in the formol percentage of the P.S.N. at the highest temperatures, suggesting that high molecular or colloidal nitrogenous substances are then being extracted or proteolysis restricted.

(10) There is a distinct restriction of amylase activity, as indicated by the extract and apparent maltose (reducing power) production, as the acidity of the mash increases to correspond with a p_H value of about 5·0 in the wort.

(11) This restriction is more marked with maltose than extract and still more marked with fermentability.

(12) The last observation corresponds with optima at increasing p_H values for extract, reducing power and fermentability, namely at about p_H 5·1, 5·3 and 5·5 respectively. p_H 5·5 is the optimum for α-amylase.

(13) The greater restriction of fermentability at higher acidities suggests that more of the reducing power is then due to unfermentable dextrins and less to maltose.

(14) The more rapid saccharification around p_H 5·5 also corresponds with the optimum for α-amylase.

(15) The buffering power of wort is increased below p_H 5·0 in the p_H zone 5–4, in which it is due largely to products of protein degradation. This corresponds with the optimum for proteolysis.

(16) The increase of buffering power in the phosphate zone extends only to about p_H 5. The p_H optimum for phytase action is at about p_H 5·2.

(17) The fall of p_H value during fermentation is greater at

more alkaline reactions. The reduction in the increase of real acidity during fermentation at lower p_H values is due to the greater production of buffers in the p_H zone 5–4.

(18) The fall of p_H value during copper boiling is greater with worts of higher p_H value.

(19) The influence of the reaction of the mash differs according to the colloidal condition or state of preparation of the substrate, that is with the modification of the malt. For example, a marked increase of extract is obtained with under-modified malts by adjusting the reaction of the mash until the p_H of the wort is about 5·15, but the increase may be very slight with a fully modified malt.

SPARGING

(450) Influence of Sparging on Wort Composition.

Sparging is intended to wash absorbed wort from the extracted grains and thereby minimise loss of extract. It is carried out by a spray of liquor at a higher temperature than that of the mash and thereby altered conditions are set up in the mash tun. The temperature gradually rises as the wort is displaced by liquor, enzymes are destroyed, buffering substances are removed and the resistant colloidal constituents of the grains are subjected to attack at a relatively high temperature by a large volume of liquor, at a reaction which may be considerably more alkaline than that which had previously prevailed. The physical effect of liquor at 167°–172° F. is sufficient to liberate unconverted starch from the matrix of protein and other material in which it was embedded. This would occur at a time when most of the starch-converting enzymes had been removed with the earlier worts, destroyed by the rising temperature or temporarily inhibited by maltose. The α-amylase, however, retains its activity at very high temperatures and any that remains, when the inhibiting effect of the high concentration of maltose is removed by dilution during sparging (Section 467), will split starch to dextrins which will not be saccharified, on account of the absence of β-amylase. This accounts for the blue or brown colour sometimes produced by addition of iodine solution to last runnings.

Starch is not the only substance extracted under these conditions. High temperature and rising alkalinity facilitate the dispersal of other colloidal substances. Among them are hemicelluloses and other polysaccharide constituents of the malt, unchanged or partly degraded proteins, tannins and other coloured substances with objectionable flavours from the husks, together with silica. The deleterious influence of carbonates in the liquor becomes marked, since, owing to the removal of buffers, the p_H

value of the liquid acting on the grains may rise to 6·0 or 6·5. If the liquor is of suitable composition or properly treated, the p_H value of the later runnings should not rise by more than one or two tenths of a unit from its original value of 5·1 or 5·2 in the strong wort of an infusion mash. This does away with one of the most potent factors that operate to change the composition of the extract. Some of the extracted substances may serve a useful purpose, but others are so objectionable that it is preferable to sacrifice a certain amount of extract, rather than risk the reduction in quality, brilliance or stability of beer entailed by their presence. The nature of these risks must be examined and consideration given to the possibility of reducing them without loss of extract through rejection of runnings at more than negligible gravities. The alternative of using the last runnings with the mashing liquor of the next brew, so that some of their unconverted constituents may be broken down in the mash tun, is not always convenient or possible. Examples by Harman and Oliver [10] of the gradual change in wort composition during sparging of an infusion mash are given in Table 193.

TABLE 193.—ANALYSES OF MASH TUN WORTS AND SPARGINGS (HARMAN AND OLIVER)

Time mins.	Brls.	Sp. gr.	Wort solids %	p_H	N as protein		Ash %		$[\alpha]_D$	Apparent maltose % on solids
					% on wort	% on solids	in wort	on solids		
Wort A										
0	20	1086·38	22·4	—	1·132	5·03	·2950	1·32	116°	67·9
26	70	1086·66	22·4	—	0·880	4·87	·2972	1·32	116	68·3
36	98	1076·30	19·8	—	0·968	4·89	·2736	1·38	115	68·3
43	115	1058·94	15·3	—	0·752	4·96	·2212	1·44	115	68·6
Second copper										
59	29	1039·68	10·3	—	0·580	5·64	·1794	1·74	110	64·7
79	70	1019·98	5·18	—	0·298	5·75	·1124	2·78	105	56·9
101	130	1008·34	2·16	—	0·152	7·04	·0742	3·43	98	48·4
136	180	1004·08	1·06	—	0·096	9·06	·0570	5·99	66	50·8
Wort B										
0	—	1077·50	20·12	5·08	0·952	4·75	·2662	1·33	116	73·8
30	27½	1082·36	21·3	5·12	1·056	4·89	·2874	1·35	115	72·8
48	55	1084·42	21·8	5·14	1·100	5·03	·3172	1·45	114	72·7
67	75	1085·08	22·2	—	1·080	4·88	·2810	1·27	114	73·2
94	100	1055·20	14·3	—	0·708	4·94	·2280	1·61	115	73·2
99	110	1049·72	12·85	5·45	0·618	4·72	·2076	1·61	114	72·2
111	120	1036·40	9·42	—	—	—	·1392	1·48	116	72·4
Second wort										
117	—	1030·70	7·95	—	0·4156	5·09	·1340	1·69	117	70·2
—	30	1005·74	1·49	—	0·140	9·40	·0598	3·67	103	48·2
—	60	1001·44	0·37	—	0·736	19·7	·0424	11·65	111	25·2

(451) Nitrogenous and Ash Constituents of Spargings.

The figures in Table 193 show a large increase in the percentages of nitrogen and ash in the total solids during sparging. Further details of qualitative changes in the protein and mineral constituents of the worts are afforded by Schild's [11] analyses in Table 194. The methods he employed for fractionation of the nitrogen at various periods during the running of a pale export lager wort are empirical, but indicate a definite progressive change in the nature of the nitrogenous substances (Section 471). This consists in a rise in the proportion of the more complex protein derivatives and a fall in that of the simpler degradation products. Changes of this kind must be regarded as disadvantageous to the protein stability of the beer, and would be increasingly so with greater proportions of spargings in the wort. The buffering over two ranges of p_H was determined by titration of worts concentrated *in vacuo* to 8% Plato and did not correspond with that of the collected worts. The increased buffering in the lower zone was due largely to the increased proportion of nitrogenous substances in the wort solids, phosphates playing a continually diminishing part in it.

TABLE 194.—CHANGES IN THE COMPOSITION OF THE EXTRACT DURING SPARGING (E. SCHILD)

Extract, Plato % Sp. gr. 20°/20° C.	16·6 1068·0	7·95 1031·6	2·48 1009·7	1·14 1004·4	0·74 1002·9
p_H value	5·80	5·95	6·22	6·34	6·49
Total N % of solids	0·8040	0·8579	1·032	1·250	1·388
Coag. N % of total N	9·05	12·74	14·09	18·67	11·30
Perm. sol. N % of total N ..	90·9	87·3	85·9	81·3	88·7
Tannin N % of total N	29·5	29·8	33·8	38·2	36·4
Phosphomolybdic N % of total N	42·4	43·3	49·0	54·8	56·0
Formol N % of total N	37·4	36·7	29·9	27·3	24·4
Ash % of solids	1·30	1·53	2·16	3·16	4·28
SiO_2 % of ash	8·0	9·3	18·3	25·8	30·4
P_2O_5 % of ash	45·3	41·7	32·8	25·8	19·8
Buffering 8% ext.					
to p_H 7·07	5·55	6·80	9·30	11·65	12·20
p_H 7·07–9·0	10·90	11·25	11·85	12·50	13·90
Apparent maltose %	69·0	72·6	72·3	68·3	66·8
Furfural value	1·30	1·06	0·81	0·79	0·91

The increase in the proportion of ash in the total solids is to be attributed to the diminution of saccharine matter and extraction by hot liquor of less soluble mineral matter that escaped extraction during mashing. The increase in silica from the husks is notice-

able, while phosphates from the malt diminish as sparging proceeds. According to Netscher [12] the extraction of silica is detrimental to the subsequent stability of the beer. Silicic acid, like other colloidal oxides, is very apt to be precipitated if the colloidal equilibrium is disturbed. This is marked in the presence of proteins, and it was found, with pasteurised beers from different breweries, that those with the highest content of silica became hazy most rapidly and gave the heaviest deposit. Its extraction is increased with alkaline sparge liquors. That used in the brew at a large Munich brewery, where Schild's analyses were made, was the City supply, partly decarbonated and slightly hardened by gypsum. Its carbonate hardness corresponded with 11·5 parts per 100,000 of $CaCO_3$ and its permanent hardness with 10·1 parts per 100,000 of gypsum. The sparge temperature was 172·4° F.

(452) Effect of Liquor Treatment on the Composition of Spargings.

TABLE 195.—INFLUENCE OF REDUCTION OF CARBONATES IN SPARGE LIQUOR (SCHREDER *et al.*)

PERCENTAGES ON WORT SOLIDS

Carbonates as $CaCO_3$	Untreated liquor 29 5 pts. 100,000		Treated liquor 17·5 pts. 100,000	
	wort	spargings	wort	spargings
p_H value	5·77	6·18	5·78	6·08
Solids gr. per 100 ml.	17·24	0·60	17·50	0·63
Apparent maltose	63·8	47·2	65·5	54·6
Protein (N × 6·25)	5·0	9·1	5·1	8·6
Ash	1·2	6·3	1·3	6·2
Sugar/non-sugar ratio	1 : 0·565	1 : 1·120	1 : 0·526	1 : 0·830
As % of total N				
Coagulable N	5·6	10·9	5·8	10·4
Proteose N	12·2	25·0	13·4	19·9
Peptone N	25·7	27·5	26·4	24·0
Polypeptide N	24·3	14·7	23·2	21·0
Formol N	32·2	21·9	31·2	24·7
SiO_2 as % of ash	13·5	30·0	9·9	22·1
P_2O_5	40·8	8·7	41·7	12·2

The composition of the worts can be made more regular during sparging by treatment of the liquor in such a way as to reduce its alkalinity. The p_H value of the runnings can be maintained at an almost constant figure by removal of the carbonates, with the result that the proportion of nitrogen and particularly of high molecular protein degradation products is reduced and that of formol titratable nitrogen increased, with material advantage to the protein stability of the beer. This is shown by analyses of decoction mash worts and spargings by Schreder, Brunner and Hampe[13] with liquor in which the carbonate hardness was reduced from 16·5 to 9·8 parts CaO per 100,000 by partial neutralisation with hydrochloric acid, the total hardness being 22 German degrees in both cases (Table 195). The p_H values of the collected worts were 5·58 and 5·48, respectively. The empirical nitrogen fractionation was based on (1) saturation of boiled and filtered wort with zinc sulphate, (2) precipitation with a solution of sodium phosphotungstate in sulphuric acid, and (3) formol titration (see Section 471).

(453) Division of Worts.

The changes in wort composition during sparging appear quite considerable, when expressed as a percentage on dry matter but the actual quantity of the latter added with a given·bulk of spargings becomes progressively less and may seem insignificant in the last runnings. Nevertheless variation in the quantity of spargings collected makes a material difference in the composition of the total worts. The figures for the first brew in Table 193 are averaged in Table 196 to show approximately the composition of worts collected in two coppers. They indicate the wide variations that may occur when the worts from a mash are run off successively into two or more coppers. In this case the second copper worts are seriously lacking in nitrogen and the percentage of apparent maltose on the wort solids is considerably less. The reduction in reducing power of the second wort may not be a measure of its relative deficiency in maltose, since a greater proportion may be due to dextrins or other substances, than in the first wort. If a light beer was made from this second copper wort, with a comparatively small quantity of the first to make up the gravity, fermentations might be expected to suffer. Though the quantity of mineral matter in the second copper is

TABLE 196.—COMPOSITION OF FIRST AND SECOND COPPER WORTS (HARMAN AND OLIVER)

Worts	Sp. gr.	Wort solids %	Nitrogen gr. in 100 ml.	Nitrogen % on wort solids	Ash gr. 100 ml.	Ash % on solids	Apparent Maltose % on solids
1st copper	1082·5	21·4	0·169	0·80	0·286	1·34	68·3
2nd copper	1020·6	5·3	0·050	0·96	0·133	2·51	55·9

much smaller than in the first, the percentage on dry solids is greater, but they would contain a relatively small proportion of mineral yeast nutrients from the malt and a greater percentage of salts from the liquor. The composition of the nitrogenous extract in the second copper wort would also be quite different from that in the first worts and might lead to defective copper break and lack of brilliance in the beer.

For the above reasons it is preferable to boil in one length, rather than divide the earlier strong worts and later weaker runnings in two or more coppers. If the capacities of the coppers do not permit of a single boil, or if it is necessary to produce worts of different gravities for a parti-gyle, the stronger worts should be divided among coppers to the best advantage. By this means a satisfactory break is secured in all the coppers, and the adverse influence of the later runnings minimised. In many ways it is advisable to hold over and boil the last runnings with the mashing liquor for the next brew, in which unconverted material would be enzymically broken down. Wort A in Table 193 was run off in two copper lengths. Wort B was a single length.

ADJUSTMENT OF MASH AND WORT REACTION

(454) Adjustment of Mash Reaction.

The principles discussed in the earlier Sections of this Chapter and illustrated by analyses of worts from mashes in which different acidity conditions prevailed can be applied in practice in circumstances in which it appears desirable to activate or check enzymic activity. An adjustment of the reaction of the mash to a p_H value of 5·1 or 5·2 is necessary to cause most of the enzymes to exercise their full activity, but strict caution has to be exercised in making such an adjustment, because it might result in restriction of the reaction of some enzymes if carried too far. It is now known that the old established methods of liquor treatment, based on removal of carbonates and addition of gypsum and calcium chloride, have, as one result, an increase in the hydrogen ion concentration of the mash. With some natural and treated liquors, no further adjustment may be necessary and the very important factor of satisfactory reaction during sparging is met. In the majority of cases, however, further steps are desirable to maintain the reaction of the mash and wort at the p_H value found to give best results with the type of beer brewed and the materials and methods employed. The methods by which this may be effected are now so well known and so readily controlled, that adjustment of reaction takes its place with adjustment of temperature and process as an important factor in maintaining regularity of wort composition.

Poulsen [14] summarised the advantages secured in practice at the Carlsberg Brewery by reduction of the p_H value of the mash from 5·4 to 5·1, by a method similar to that described in Section 457, as follows, but emphasised the point that restriction of saccharification was liable to occur if the reaction was brought to a p_H value of 5·0 or lower.

(1) Paler beer.

(2) Brilliancy improved.

(3) Better break on boiling and cooling.

(4) Beer clarifies more quickly and completely during storage.

(5) Beer flavour better. There is no acid flavour under the conditions adopted and the harsh bitter flavour due to carbonates in the liquor is avoided. The hop flavour is milder and more delicate.

(6) Beer remains bright for a longer period after pasteurisation.

(7) Gas retention of bottled beer improved, with less foaming over when the bottles are opened.

(8) Increase of extract, particularly if the malt is under-modified.

(455) Adjustment of Wort Reaction.

Stimulation of enzymic activity by adjustment of the reaction of the mash increases the buffering of the wort by products of protein breakdown and inorganic phosphates. This results in greater resistance to the fall in p_H value that always occurs during fermentation, so that the reaction of the beer at racking may be only very slightly more acid than that of beer from an untreated mash. It may, indeed, prevent attainment of a sufficiently low p_H value at racking to assure the biological stability of the beer. The most desirable final p_H value varies with different beers, but is generally between 4·2 and 3·9 with English beers (Parsons[15]). A reduction in the p_H value at racking, should this be found desirable, can be secured by slight acidification of the wort in the copper, instead of or in addition to treatment of the mash. The different effects of mash and wort acidification on the p_H of the finished beer are shown in Table 197. A reduction of the p_H value of these worts by 0·3 appeared to be necessary to reduce that at racking by 0·1. Treatment in the copper has other advantages, in that it may be more effective in bringing the wort reaction to that most favourable to protein coagulation and in restricting the formation of very bitter compounds of the hop resins with alkaline salts (Section 492).

(456) Methods of Adjusting Reaction.

Some natural gypseous liquors bring the p_H value of a malt mash very close to 5·2, but treatments involving removal of carbonates and gypsum additions are not always quite effective in this respect and it is frequently desirable to supplement them by a slight acidification of the mash or wort. Several methods are available for this purpose, but they should all be considered as supplementary to liquor treatment and not as alternatives if it is desired to reduce the p_H value of the mash or wort by more than 0·2 or 0·3. They include addition of small quantities of lactic acid, preferably by the use of biologically acidified wort containing about 1% of that acid, or by use in the grist of 2 to 5% of biologically acidified malt (Vol. I, Section 216). Addition of acid to the liquor or mash is not permitted in Germany, unless it is in the form of biologically acidified wort or malt, both of which are becoming increasingly adopted. Addition of mineral acids to the mash or wort is not to be advised. Their salts lack the buffering power of lactates, so that the resultant fall in p_H value may be much more abrupt.

Biological acidification of the mash has a different effect from decarbonation of the liquor or treatment of the latter with mineral acid, in that the carbonates appear as lactates in the wort and the last runnings are more alkaline than with decarbonated liquor. Carbonates should always be removed from the sparge liquor, if present in any quantity. Reduction of the p_H value of the wort has, in itself, very little effect on the fermentations or final attenuations, but improvements in fermentation are frequently noted with Enzymic malt and must be attributed to other reasons connected with the composition of the malt (Section 682).

(457) Biological Adjustment of the Reaction of the Mash.

This process involves addition to the main mash of a suitable quantity of a specially prepared soured mash, in order to reduce the p_H value of the former to the required extent. *Lactobacillus delbrückii* is the most suitable organism for souring the small mash. It produces most lactic acid between 107·5° and 111° F. and grows quite actively at temperatures as high as 124°–126° F. This is an important factor in its successful use, since practically pure cultures can be obtained by growth at temperatures fatal to most other organisms likely to be present. Pure cultures of *L. delbrückii* can be purchased but, if no convenient source of supply is at hand, they can be obtained by adding a little distillery yeast, in which it almost always occurs, to a mixture of malt and water and incubating at 124° F.

A covered, steam-jacketed copper or, preferably, stainless steel vessel is generally used for preparation of the acid mash, but an ordinary wooden cask, suitably insulated or heated, can be used for experimental purposes. A great deal of trouble and the expense of a thermostat can be avoided by placing the cask near to boilers or in some place where the temperature of its contents can be maintained at 107°–117° F. This range of temperature is preferred to 122°–124°, because the latter is rather too high for maximum acid production and there is little fear of infection above 107° F.

Mash tun wort at a specific gravity of about 1075, cooled to 117° F., may be inoculated with about 1% of its volume of a pure culture of the bacillus and incubated for 20 or 24 hours at 117°. At the end of this time, its acidity should be about 1%, calculated as lactic acid. The acid content should always be controlled by titration with N/10 NaOH against phenol-phthalein. Its p_H value does not give the necessary information, as this will vary, for any given lactic acid content, with the buffering of the wort, but may be about 3·2 with 1% of lactic acid. 1% of the soured wort may be retained for inoculating the next charge, when the bulk is transferred to the main mash.

The amount of soured wort required to reduce the p_H of the main mash by 0·2 or 0·3 will vary with the materials and liquor employed, and must be carefully controlled by determination of the p_H of the mash. It is always advisable to proceed with great care, gradually increasing the acidity of the main mash until the best beer is obtained. Thus the quantity of acidified wort added should not at first reduce the p_H of the main mash below 5·2. As an example of the quantity of wort with a lactic acid content of 1% required to reduce the p_H value from 5·4 to 5·1, Poulsen stated that ·33 gallon was used with a mash made from 20 cwt. of malt and a liquor containing in parts per 100,000

CaO	..	13·2
MgO	..	2·3
CO_2	..	11·0
SO_3	..	2·4

2 barrels were required for a 130-barrel brew in another case.

The required quantity of acidified wort is blown to the main mash by air pressure and well mixed with it, previous to raising the temperature above 122°, in the case of a decoction mash. 5 to 10 gallons of the wort may then be run from the main mash to the small vessel to start the culture for the next day's mash. Immediately after this, the temperature of the main mash is raised as usual, ensuring sterilisation. This occurs very rapidly, if the culture is added to an infusion mash at ordinary mashing temperatures, so that a propagation from the main mash is impossible, for which reason continuous cultures are maintained by retaining some of the soured mash.

(458) Use of Biologically Acidified Malt.

Biological acidification of the mash, as described in the last Section, is troublesome and requires constant supervision. For this reason many brewers, though recognising its utility, have hesitated to take advantage of it. The difficulties are entirely overcome by the use of a small proportion of the biologically acidified Enzymic malt, made by Dixon's process. 3 to 5% of the malt can be blended with the grist, thereby raising the acidity of the mash, or a cold water extract can be added to the copper, if it is desired to increase the acidity at that stage. The lactic acid content of the malt can be varied in the maltings, so that increased control in the brewery is possible. The grade containing 2% of lactic acid yields a 10% laboratory wort with a p_H value of 4·4 or, mixed at the rate of 5% with an ordinary English malt in the Institute of Brewing extract mash, reduces the p_H value of the wort by about 0·2.

When it is desired to adjust the reaction of the wort, instead of or in addition to that of the mash, this can be readily effected by adding a suitable quantity of a cold water extract of the biologically acidified malt to the copper. This extract can be made by mashing a quarter of the malt in four barrels of cold liquor. The mash is stirred for about an hour, after which the grains are allowed to settle and the extract syphoned off for use. The grains may be used in a brew. Alternatively a small mash can be made at about 150° F. and the wort used for acidification.

(459) Effects of Adjustment of Reactions of Mash or Wort.

Three examples from published analyses are given to illustrate the influence of adjustment of the reaction of the mash or wort on the composition of the wort and beer. The first, by Kolbach, Haussmann and Wilharm,[16] is taken from a series of trials at three breweries and gives the results obtained when varying quantities of biologically soured wort, prepared by the method described in Section 457, were added (*a*) to the first boiling mash and, with this, to the main mash, raising the temperature of the latter from 125·6° to 158° F. This would increase the acidity of the mash during the saccharification period, but not during the protein rest which preceded it; (*b*) as the wort was collecting in the copper; and (*c*) part of the soured wort was added to the mash and part to the wort in the copper. The analytical results are all adjusted to an extract of 12% (1048·4), from which the three brews varied only very slightly. The liquor at this brewery contained 17 parts per 100,000 of $CaCO_3$ and 7 parts per 100,000 of $CaSO_4$.

TABLE 197.—EFFECTS OF BIOLOGICAL ACIDIFICATION OF (A) MASH, (B) WORT, (C) MASH AND WORT ON THE COMPOSITION OF WORTS AND BEERS (KOLBACH *et al.*)

ANALYSES IN MGMS. PER 100 ML. WORT OR BEER 12% EXTRACT

	A			B			C		
*Kg. Lactic acid in mash..	0	0·52	0·75	—	—	—	0·23	0·46	0·75
„ in wort ..	—	—	—	0·21	0·42	0·66	0·38	0·45	0·44
p_H	5·60	5·35	5·28	5·48	5·34	5·27	5·31	5·12	5·09
Buffering p_H 7·07–4·27 ..	15·0	17·4	17·6	15·00	15·26	15·62	16·31	17·37	17·96
P.S.N. mgm.	91·1	107·0	104·7	92·7	93·6	92·3	98·6	106·0	105·1
„ % of malt N..	35·2	41·3	40·4	35·7	36·2	35·6	38·1	40·9	40·6
Formol N mgm.	30·3	34·8	34·9	30·9	32·2	30·5	32·3	35·7	34·5
„ % P.S.N. ..	33·2	32·5	33·3	33·3	34·4	33·0	32·8	33·7	32·9
Phosphorus mgm. ..	37·0	41·7	43·1	37·2	38·5	38·5	39·9	42·9	42·4
Colour ml. N/10 iodine ..	1·30	1·11	1·00	0·99	1·12	1·00	1·01	0·92	0·92
Beer									
p_H	4·54	4·57	4·55	4·50	4·49	4·45	4·50	4·49	4·46
Buffering p_H 7·07–4·27 ..	16·5	18·5	18·3	16·47	16·83	17·24	17·22	18·60	18·93
P.S.N. mgm.	71·6	85·8	86·1	75·0	77·4	76·8	78·5	85·4	83·9
Formol N mgm.	22·5	26·3	25·4	23·1	24·0	25·0	24·6	25·5	25·2
„ % P.S.N. ..	31·4	30·7	29·5	30·8	31·0	32·5	31·3	29·9	30·0
Hop resins mgm.	79	75	70	82	76	70	—	—	—
Colour ml. N/10 iodine ..	1·00	0·78	0·80	0·89	0·87	0·74	0·82	0·75	0·67
Apparent Atten. % ..	74·1	74·2	74·7	75·0	73·5	74·5	75·4	75·9	74·7
Limit Atten. % ..	82·8	80·8	81·4	81·9	82·1	82·0	82·5	81·6	80·9

* Kilograms of lactic acid per 50 kg. malt, added in the form of soured mash.

The results recorded in Table 198 were obtained by Lüers [17] in comparable brews of lager beer, in some of which 4% of Enzymic malt was used in the grist, while in others a cold water extract of the same quantity was added to the wort collected in the copper. The liquor composition or treatments and the malt additions were as follows.

Brew No. 1.—Normal brew with softened and treated liquor containing 9·4 parts per 100,000 of gypsum and 5·6 parts per 100,000 of calcium carbonate.

Brew No. 2.—With untreated liquor, containing 27 parts per 100,000 of calcium carbonate.

Brew No. 3.—Same liquor as No. 2, but with 4% Enzymic malt in the grist.

Brew No. 4.—Same liquor as No. 2, but with the cold water extract of 4% Enzymic malt in the copper.

Brew No. 5.—Softened and treated liquor, containing 9·4 parts per 100,000 of gypsum and 3·6 parts per 100,000 of calcium carbonate, with the extract of 4% Enzymic malt in the copper. The nitrogen fractionation is described in Section 471.

TABLE 198.—ANALYSES OF WORTS AND BEERS FROM BREWS WITH AND WITHOUT BIOLOGICALLY ACIDIFIED MALT (H. LÜERS).

Brew	Total N mgm. 100 g.	N % of total N				Acidity 100 g.1048 wort. ml. N/10 NaOH			p_H	Limit. atten.
		Tannin N	$MgSO_4$ N	Adsorbed active C	Formol N	Neutral red	Phenol-phthalein	Total		
1 Wort	100·2	21·1	22·0	54·8	36·2	0·86	1·91	2·77	5·73	67·90
Beer	80·2	—	21·1	59·6	30·9	1·38	1·68	3·06	4·58	—
2 Wort	94·6	22·9	23·4	54·5	36·5	0·84	1·68	2·52	5·91	67·67
Beer	72·1	—	22·8	58·5	31·1	1·20	1·50	2·70	4·87	—
3 Wort	100·9	19·4	20·6	53·9	35·1	1·00	1·81	2·81	5·71	67·06
Beer	73·4	—	21·5	57·4	30·9	1·43	1·58	3·01	4·95	—
4 Wort	97·4	20·0	21·6	53·5	35·9	0·95	1·72	2·67	5·78	66·7
Beer	77·5	—	20·8	58·6	31·0	1·32	1·61	2·93	4·64	—
5 Wort	101·8	19·3	21·0	52·9	35·8	1·10	1·87	2·97	5·56	66·6
Beer	82·6	—	20·0	54·6	33·3	1·57	1·85	3·42	4·62	—

The figures in Table 199 are typical of the increased extracts usually obtained by an appropriate increase in the hydrogen ion concentration of the mash. They are from analyses by F. Windisch [18] of worts of three types of lager brewed with and without Enzymic malt in the grist.

TABLE 199.—INFLUENCE OF MASH REACTION ON EXTRACT (F. WINDISCH) BREWS WITH AND WITHOUT ENZYMIC MALT

	Export		Munich		Pilsen	
	Control	With 5% Enzymic	Control	With 5% Enzymic	Control	With 3% Enzymic
p_H of wort	5·81	5·58	5·67	5·51	5·85	5·62
p_H of beer	4·52	4·47	4·40	4·28	4·52	4·42
Buffering, p_H 7·07–4·27	15·76	16·79	26·42	29·05	16·11	17·72
Extract % Plato ..	73·69	74·72	71·12	72·57	74·06	75·00
Extract lb. per quarter	92·1	93·4	88·9	90·6	92·6	93·7

The results obtained in these brews correspond with those of the experimental mashes. The most significant changes in wort composition produced by acidification of the mash are increases in the nitrogen and phosphate content, associated with greater buffering and extract. Little, if any, change is shown in the nature of the nitrogen compounds in Tables 197 and 198. The formol nitrogen increased *pari passu* with the permanently soluble nitrogen. It is possible that improvements in methods of nitrogen fractionation will eventually show that changes do occur to account for the greater stability of the beers recorded in each case. This may, however, be due to activation of other enzymic changes, of which the analyses give no hint, and to the improved copper breaks that were noted.

The figures for permanently soluble nitrogen as a percentage of malt nitrogen in Table 197 indicate that the increased proteolytic activity associated with reduced p_H value has an effect on wort composition analogous to that produced by greater modification of the malt. This emphasises the special usefulness of mash acidification with under-modified malts.

The different effects of acidification of the mash or copper wort are shown by the figures in Table 198. Use of 4% Enzymic malt in the grist of brew No. 3 had practically the same effect on the p_H value of the wort as the liquor treatment of No. 1, No. 2 being the control with untreated liquor. Addition in the copper of the cold water extract from the same quantity of Enzymic malt, brew No. 4, reduced the p_H value of the wort and increased its titratable acidity almost as much as in brew No. 3. The greatest increase in acidity and nitrogen content was produced in brew No. 5, under combined action of liquor treatment and acidification in the copper. Reduction in the quantity of hop resins extracted by chloroform from the more acid worts in Table 197 corresponds with greater softness of flavour of the beers. Acidification in the copper produced the best effects on beer flavour.

The benefit obtainable by acidification varies under different circumstances, according to the extent to which the effects it produces are opposed by carbonates in the liquor or by under-modification of the malt. Improvement in the flavour and stability of the beer may be anticipated whenever defects due to these causes are known to exist, and are obtained, to some extent, in a great many, if not the majority of cases. The flavour of beer is generally rather cleaner and its stability considerably improved. No comparable analyses of infusion mash worts have been published. Greater reliance is placed on liquor treatment to give the desired p_H value in the mash, and more fully modified malts reduce the necessity for activation of enzyme action but, even so, the small increase of acidity produced has been found to be of definite value. The degree of acidification required and the best place to apply it must be found by trial. A p_H value of 5·1–5·2 is generally found most advantageous for top fermentation beers, mashed by infusion methods, and for pale bottom fermentation beers with a decoction mash and proportion of raw grain. For other lager beers a p_H value of 5·4 is found to be more suitable and a higher p_H value in the mash is generally desirable in dark than pale beers.

(460) Summary.

The reaction of the mash is, with mashing process and temperature, one of the most important operative factors over which the brewer has control in determining the composition of the wort. The p_H value of a malt mash with distilled water is generally about p_H 5·8, which is considerably higher than the optimum for the activity of most enzymes. A carbonate liquor gives a wort that may have a p_H value of 6·0 or more, but the ordinary methods of liquor treatment by removal of carbonates and addition of gypsum make it possible to reduce the p_H value of the mash until that of the wort is about 5·1 or 5·2. This is very close to the optimum for extract production and a very useful mean value for the operation of the starch-converting, proteolytic and phosphate-producing enzymes. It is frequently found advantageous in practice slightly to increase the acidity of the mash to overcome the adverse influence of small quantities of carbonate left in the liquor after treatment, or to stimulate the activity of enzymes, particularly when the malts are somewhat under-modified. The reaction should not, however, be brought below p_H 5·0, as this tends to restrict the diastatic enzymes and reduces the fermentability of the wort.

The final adjustment of the reaction of the mash can be effected by addition of biologically acidified wort to the mash or by the use of biologically acidified Enzymic malt in the grist. The resulting stimulation of the enzymes increases the permanently soluble nitrogen and phosphate content of the wort. In practice it very often produces a more brilliant and stable beer, with better flavour. The increase of buffers in the wort, however, restricts the fall of p_H value during fermentation, so that the beer may not attain the p_H of 4·0–4·2 at racking required to ensure its biological stability. It is consequently in some cases advisable to acidify the wort in the copper, rather than the mash. In this case there is no stimulation of enzymic activity and no increase in the soluble nitrogen or phosphates. The beer will then attain a low p_H value at racking. In addition, the reduced p_H value of the wort in the copper prevents excessive formation of very bitter compounds of the resins and alkalis and so leads to a softer flavoured beer. In other cases it is best to acidify both mash and wort slightly. The circumstances in respect of material, mashing process and beer character are so varied that the most satisfactory method and degree of acidification should be found by trial. In all-malt lager beers a rather higher p_H value appears to be desirable than in top fermentation ales or lager beers brewed with a proportion of unmalted cereals.

The p_H value of the later runnings from the mash tun are very liable to rise to 6·0 or higher, unless carbonates are removed from the liquor. This, with the increased temperature of the sparge liquor, causes extraction of undesirable colloidal substances from the malt at a time when active enzymes no longer exist to break them down to a truly soluble state. The later runnings, which differ materially in composition from the strong worts, should not be boiled alone, but blended with the stronger worts in each copper, in order that the break may not be adversely affected. Poor break and excess of undesirable substances extracted from the husks or resistant starch and proteins of the malt during sparging lead to lack of brilliance in the beer and an early tendency to haze formation.

The reaction of an ordinary malt mash and that of the wort are determined by the buffers present, particularly by phosphates extracted from the malt. Variations produced by different liquors are attributed mainly to reactions between the sulphates and carbonates of the liquor and the primary and secondary phosphates. The influence of lactic acid bacteria is generally regarded as of minor significance in an infusion mash. Some of these bacteria, invariably present on malt, can, however, withstand comparatively high mashing temperatures, and Pierre[19] contends that the lactic acid they produce has an important function. He holds that it does not persist as lactic acid, but increases the acidity of the mash and wort by converting neutral and alkaline secondary and tertiary phosphates to acid primary phosphates.

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CHAPTER XXVII

STARCH CONVERSION AND PROTEOLYSIS IN THE MASH

THE CARBOHYDRATES OF WORT

(461) The Problem of Starch Conversion.

Three outstanding aspects of the problem of starch conversion are illustrated in a diagram, Fig. 115, reproduced from Hanes'[1] review of the present state of knowledge of the action of amylases in relation with the structure of starch. These are concerned with (*a*) the nature of starch itself, (*b*) the agents of its breakdown, and (*c*) the dextrins. Hanes' schema was put forward as an attempt to summarise (*a*) certain fairly well established features, e.g., the mode of action of β-amylase, (*b*) certain outstanding problems of importance, e.g., whether or not a specialised liquefying enzyme exists, and the mode of attack and the nature of the products of α-amylase.

All native starches are generally believed to be composed ultimately of molecular units of similar if not identical structure. This is represented, according to the generally accepted view, by a chain of 30 links, each of which is a glucose residue (Vol. I, Section 106). Three of these molecular units are shown in the simplest manner as straight chains in the diagram, but this is not intended to preclude the possibility that they might be more accurately represented by some form of coil or spiral. This view of the " constitution of starch " is based primarily on the chemical investigations of Haworth and his colleagues, but is strongly supported by Blom, Bak and Braae's[2] finding that the products of enzymic breakdown under different conditions always represent almost exactly one-fifteenth or some multiple of one-fifteenth of the weight of starch acted upon. It is thus reasonable to conclude that the molecular unit consists of 15 maltose or 30 glucose residues or some multiple of these. Our knowledge of the constitution of starch and its hydrolysis is based mainly on the study of potato starch, but Baker and Hulton[3] have recently proved by preparation of an identical enzymic conversion product from purified potato and malt starches under identical experimental conditions that the general use of the more readily available pure potato starch for purposes of investigation was justified, and that the results could be applied with considerable confidence to malt starch.

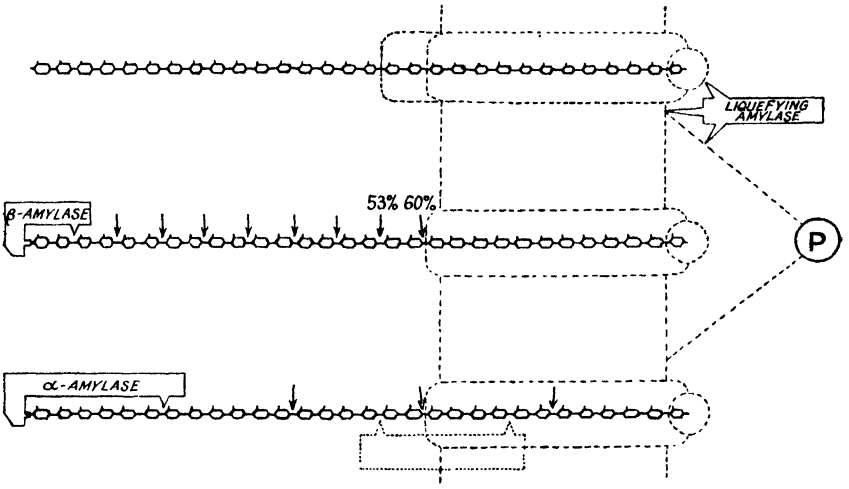

FIG. 115

DIAGRAM ILLUSTRATING THE ENZYMIC BREAKDOWN OF STARCH (C. S. HANES)

The hypothetical molecular units of starch are large, as molecules go, approaching the limit of ultramicroscopical visibility (Vol. I, Section 80). They are consequently referred to as macromolecules, but they must be distinguished from the colloidal particles or micelles of starch, in which a large number are bound together in varying degrees of aggregation, so that their resistance to enzyme attack is different. Amylose and amylopectin, amyloamylose and erythroamylose are examples of physically different fractions separated from starch. No indication of these differences could be given in a diagram restricted to three macromolecules, or of the granules constructed of a large number of colloidal particles, according to the plan characteristic of the plant in which they were formed, but the aggregation of molecular units is suggested by the dotted lines binding together 12 links of the chains at their aldehyde ends. The units are also joined by a linkage in which phosphorus takes part.

The identification of α- and β-amylases and their separation from malt amylase (Vol. I, Section 137) has provided means to study the individual activities of these enzymes and the results obtained during the last few years have thrown new light on the mechanism of starch conversion. The claim that amylophosphatase is responsible for breaking the linkage in which phosphorus is concerned and takes part in the liquefaction of starch is not accepted by some investigators. The existence of an enzyme of this type in aqueous extracts of barley appears, however, to be supported by the finding[4] that it is possible entirely to destroy the β-amylase by treatment with dilute iodine under controlled conditions and leave a thermolabile catalyst which brings about progressive decrease in the viscosity of starch paste, thus apparently acting as a liquefying enzyme, without degrading the molecular units of the original starch. Waldschmidt-Leitz and Mayer (Section 137) claimed that the optimum p_H for its activity was 5·6 and that the product contained no phosphorus, had an $[\alpha]_D$ of 191·5° and a reducing value, with hypoiodite, of R 5·6, corresponding with a chain length of 30–40 glucose residues.

A liquefying enzyme of this type is shown in the diagram, inducing rupture of the bonds connecting three macromolecules, liberating phosphorus and transforming the terminating aldehyde groups to the reducing form. α- and β-amylases are shown in spatial relation with the links of the chains that they split off and hydrolyse. Distinct parts of the β-amylase are indicated in union with a terminal glucose unit and with a grouping near the second link from it. A continuance of its action on succeeding pairs of links in the chain results in cleavage of successive maltose molecules until its action is arrested when 60 per cent. of the starch is hydrolysed. The invariable cessation of hydrolysis by α- or β-amylase before the whole of the starch is converted suggests that the enzyme meets resistance at some part of the chain. The nature of this resistance is unknown, but it suggests some abnormality of structure in the macromolecules. It may, in the case of β-amylase, be due to aggregation of parts of a number of macromolecules, as indicated in the Figure. α-amylase is shown attacking another macromolecule at its non-aldehydic end and some grouping adjoining the linkage between the 6th and 7th glucose residues. It thereby liberates a reducing dextrin molecule of 6 glucose units. An alternative mode of attack is shown in ghost form.

The breakdown products of a substance as lacking in homogeneity as natural starches, might be expected to be correspondingly variable in composition, and the dextrins have been well described as occupying one of the darkest corners in organic chemistry. Their uncrystallisable and colloidal character makes their isolation very difficult. The usual method of fractional precipitation or extraction in alcohols of different strength is not altogether satisfactory (Baker and Hulton[5]). It is based on the greater solubility of the simpler dextrins and increasing resistance of the more complex to solution in stronger alcohols, but the isolation of sharp or homogeneous fractions is limited by insufficient differences in solubility and by the tendency to adsorption of one substance on another, maltose on the dextrins, for example. It is also difficult to establish the homogeneity of the fractions, though evidence of individuality can be obtained by diffusion unchanged through membranes which retain more complex substances and pass the simpler. Myrbäck[6] contends that the "limit dextrins" or ultimate dextrin residues of starch conversions are not homogeneous products. He agrees that the greater part of the starch unit is constructed according to the Haworth model, but that it also includes abnormal parts which are not attackable by the enzymes. These vary in structure and it is they that remain as the limit dextrins. An insoluble residue from the starch also always remains after conversion.

Many dextrin fractions have, however, been separated and characterised by their optical rotatory and reducing powers. Lack of uniformity in methods of analysis and use of different solution divisors, with the absence of any generally accepted nomenclature, make it difficult to compare the results of different investigators. These, with heterogeneity in the dextrin fractions and use of indefinite enzyme preparations, have led to the different names and varying analytical constants ascribed to what are evidently very similar fractions and, in some cases, probably identical, though unequally purified, individual dextrins. Blom, Bak and Braae have suggested a systematic nomenclature which avoids the present confusion by denoting each dextrin by the enzyme concerned in its production and the percentage of starch broken down. Examples are given in Tables 202 and 204.

(462) Products of Starch Conversion.

Much of the data incorporated in Fig. 115 is the result of quite recent investigations, but some of the earlier work, in which chemists at the Burton breweries took an active part, is of more than historical interest. Though their conclusions in regard to the structure of starch have had to give way to those of the Birmingham school under Haworth, the pioneering work on the polarimetry and reducing power of its conversion products has remained the basis of wort analysis, and the theories on the effects of temperature on starch conversion have had an immense influence on brewing practice. It was soon recognised that the quantity of dextrins could be increased and that of maltose reduced by carrying out the conversions at moderately high temperatures or with cold water extracts of malt or diastase preparations, previously heated to temperatures above 140° F., that is with "restricted diastase." Brown and Heron[7] (1879) assigned the formula $10(C_{12}H_{20}O_{10})$ to soluble starch and pictured its conversion in stages, in each of which one ($C_{12}H_{20}O_{10}$) group was split off and hydrolysed to maltose, leaving a series of polymeric dextrins of continually decreasing molecular weight. The first two of these were "erythrodextrins" that gave a red colour with iodine solution, the remainder were "achroodextrins" that gave no colour reaction. All conversions between 104° and 140° F. came speedily to a point of equilibrium, at which four-fifths of the starch was converted to maltose and one-fifth remained as "stable dextrin." Beyond this, conversion was very slow. The dextrins formed at higher temperatures were not homogeneous, and erythrodextrins separated out first on fractional precipitation.

This conception foreshadows in some measure the modern view of starch conversion by β-amylase, but it soon became clear that it was inadequate to explain the conversion of starch in the mash tun. In the same year Herzfeld[8] had come to the conclusion that above 149° F. only erythrodextrin and a substance soluble in dilute alcohol but insoluble in 90 per cent. alcohol were formed, together with maltose. The new substance had all the optical and reducing properties of a mixture of two parts of dextrin and one of maltose, but differed from "stable dextrin" in that it was completely hydrolysed to maltose by malt extract at 140° F. It was probably only obtained in a rather impure form, but was given the name of "maltodextrin."

Among the objections to simpler conceptions that regarded starch conversion as consisting in its breakdown to maltose and one dextrin in varying proportions, as might have been suspected from Brown and Morris'[9] (1885) finding that a certain relation always subsisted between the cupric reducing power and specific rotations of the hydrolysis products, were the difficulty experienced in crystallising the "maltose" or fermenting it entirely from conversions made above 140° F. and the invariable occurrence in such conversions of a body corresponding with Herzfeld's maltodextrin, which accounted for part of the "apparent maltose." The "law of definite relation" was subsequently (1897) formulated by Brown, Morris and Millar[10] as $[\alpha]_D = 202 - 0{\cdot}64R$, on the basis of the constants $R = 0$, $[\alpha]_D = 202°$ and $R = 100$, $[\alpha]_D = 138°$ for soluble starch and maltose, respectively.

In 1889, Brown and Morris[11] met the difficulties by their "Maltodextrin theory," which laid the foundations of a scientific control of mashing and explained in an apparently satisfactory manner the main features of primary and secondary fermentation. They attributed the formula $5(C_{12}H_{20}O_{10})_{20}$ to starch, and regarded it as consisting of five complex dextrin or "amylin" groups, of which four were arranged around the fifth. The first stage in diastatic conversion consisted in liberation of the four outer groups, leaving the central nucleus as "stable dextrin." The liberated amylin groups would be completely hydrolysed to maltose at lower temperatures, but above 140° F. the conversion would only be partial, resulting in the production of "amyloïns" in which fewer of the constituent $C_{12}H_{20}O_{10}$ groups were hydrolysed to maltose as the temperature increased. These were called "high type maltodextrins" or "low type maltodextrins," according as they contained a greater or smaller proportion of unchanged dextrin and were less or more readily hydrolysed to maltose and fermented. This theory of a series of maltodextrins, with the general formula $(C_{12}H_{22}O_{11})_n(C_{12}H_{20}O_{10})_{20-n}$, is now discredited. They also believed that the hydrolysis of amyloïns was accompanied by splitting of some of the large original groups to smaller aggregations of which the "maltodextrin" $(C_{12}H_{22}O_{11})(C_{12}H_{20}O_{10})_2$ was typical and existed in all high angle conversions. This compound is now more generally regarded as reducing dextrin, containing the six glucose residues characteristic of conversions by α-amylase.

Baker and Hulton[12] have recently re-examined the products obtained by converting potato starch with precipitated malt amylase at various temperatures, and came to the conclusion that they were not so complicated or numerous as formerly believed. They found only maltose and a "limit dextrin" in conversions made between 60° and 122° F. At 150° and 165° F. maltose, "maltodextrin" and "limit dextrin" were obtained, the quantity of maltose being less and that of "maltodextrin" greater at the higher temperature. The possible existence of other substances in small quantity was not excluded. Retention of the term "maltodextrin" by these authors has no structural significance, analogous to that attributed by Brown and Morris to their series of high and low type maltodextrins, but the product was apparently identical with that mentioned at the end of the last paragraph. When dried at 212° F., "maltodextrin" is a white non-hygroscopic amorphous powder, with a slight but distinctly sweet flavour. An identical product was obtained from malt starch, the two products having constants of $[\alpha]_{D3\cdot93}$ 179·7°, $R_{3\cdot93}$ 36·6 and $[\alpha]_{D3\cdot93}$ 178·2°, $R_{3\cdot93}$ 34·7, respectively. Baker and Hulton proved that it was an entity and not a mixture of closely allied bodies by failure of repeated attempts to fractionat it, and by finding that it dialysed unchanged through cellophane membranes. They considered it to be an intermediate produce in starch conversions, its absence in those made below 122° Ft being attributed to immediate hydrolysis to maltose. Its. production is presumably due to α-amylase and its further hydrolysis to β-amylase. Its formation and survival were held to be primarily a function of temperature and not of duration or extent of conversion, its existence among the products at higher temperatures being due to restriction of the saccharifying β-amylase. It was found to be unfermentable by *S. cerevisiae*, *S. festinans*, *S. intermedius* and *S. ellipsoideus*. The "limit dextrin" had constants approximating to $[\alpha]_{D3\cdot 93}$ 185°–190°, $R_{3\cdot 93}$ 5–10 and appeared to be of the same composition at whatever temperature the conversion was made. It was degradable to maltose by malt amylase at 130° F. but, like "maltodextrin," not completely. It gave no colouration with iodine and no glucose was detected in its conversion products.

Evidence is given in Table 200 of the invariable production of "maltodextrin" by heat-restricted amylase or in high temperature conversions, by examples of apparently identical products isolated by different authors. Another dextrin fraction with constants about R 40–45, $[\alpha]_D$ 170° has also frequently been isolated and possibly results from further enzyme action on the maltodextrin. It is uncertain whether this is to be regarded as an entity or mixture, but the regularity of the constants obtained by different authors suggests that it is an individual dextrin. Hanes has tabulated the products as Dextrins A and B types. The cryoscopically determined molecular weights can only be approximate, on account of the very small fall in freezing point. That of $(C_6H_{10}O_5)_6H_2O$ is 990 and that of $(C_6H_{10}O_5)_4H_2O$ is 666.

TABLE 200.—DEXTRIN FRACTIONS FROM STARCH CONVERSIONS WITH HEAT-RESTRICTED DIASTASE

Name	Authors	Conversion Temp. F.	R	$[\alpha]_D$	Molecular weight cryoscopic
Type A dextrins.					
Maltodextrin ..	Brown and Morris (1885) ..	140°–145°	34·5	180	965
Achroodextrin 2	Lintner and Düll (1894) ..	—	34·5	183	—
Maltodextrin α ..	Ling and Baker (1897) ..	158°	32·8	180	926
Limit dextrin 2	Syniewski (1902)	170°	30	180	1040
Maltodextrin ..	Baker and Hulton (1937)..	160°	36·6	179·7	—
Type B dextrins.					
Achroodextrin 3	Prior (1897)	—	42·5	171·1	640
Maltodextrin β ..	Ling and Baker (1897) ..	158°	43	171·6	670
γ-maltodextrin ..	Syniewski (1902)	—	42·7	172·2	666

(463) Activities of Purified α- and β-Amylases.

Investigation of starch conversion by purified preparations of the individual amylases is complicated by the difficulty of separating them from their natural sources and uncertainty as to the homogeneity of fractions. β-amylase is usually obtained from barley, but the infusions may also contain a small quantity of α-amylase and also, possibly, a third liquefying enzyme or amylophosphatase. The commercial bacterial preparation "superclastase", and Taka-amylase are substantially, but not necessarily entirely, α-amylase. Both α- and β-amylase can be prepared from malt infusions by Ohlsson's methods (Vol. I, Section 137), which can also be used for purifying α- and β-amylases from other sources. It is also necessary to make sure that malt preparations do not contain maltase.

The actions of α- and β-amylase overlap in each of the three processes by which the progress of starch conversion is followed or amylase activities are measured. These processes result in (1) increase in the reducing power of the conversion liquid, (2) reduction of the viscosity of starch paste, and (3) change in the colour produced by iodine solution. Nevertheless, the change in reducing power promoted by malt diastase is due mainly to the saccharogenic or β-amylase, its saccharifying activity being very many times greater than that of the dextrinogenic or α-amylase of malt. The activity measured by the Lintner and similar methods is thus largely that of β-amylase. Reduction of viscosity is due mainly to α-amylase, and measurements of enzyme activity of malt extracts based on it refer substantially to α-amylase. The latter enzyme rapidly induces those changes that result in cessation of the colour reaction with iodine, while β-amylase in normal conversions never breaks all the linkages to which the blue colour is due.

Gradual destruction of α-amylase during its isolation in a solution of malt amylase by Ohlsson's method is shown in Table 201 by Blom, Bak and Braae,[13] although these authors used temperatures of 140° and 149° F., instead of 158°, as proposed by Ohlsson to destroy the β-amylase. The determinations of enzymic activity by reducing power, reduction of viscosity and iodine colour after different periods of time at these temperatures show, by the constancy of the relation between any pair of these figures, that only one enzyme was present in the solution after 1 hour at 140° F. The reduction in saccharifying activity corresponds with the destruction of β-amylase, while the comparatively small reduction in liquefying power shows that very little α-amylase was destroyed in an hour at 140° F. The authors concluded that the enzymic activities of malt amylase could be accounted for by the presence of α- and β-amylase only, and that the existence of another liquefying enzyme was unproven. The same authors found that α-amylase of malt infusions was completely destroyed in 4 hours at 68° F. and p_H 4·2, with little detriment to the β-amylase activity, but that the latter was gradually reduced by keeping the solution for longer periods under these conditions.

TABLE 201.—ISOLATION OF α-AMYLASE FROM MALT AMYLASE SOLUTION BY HEAT AT p_H 7·0 (BLOM, BAK AND BRAAE)
(Relative activities per 1 gr. dry malt)

Treatment	Saccharifying (S)	Liquefying (L)	Iodine (I)	$\frac{S}{L}$	$\frac{S}{I}$	$\frac{L}{I}$
Original solution ..	73·3	5050	64·0	0·0145	1·15	79
140° F. 20 minutes	18·5	4970	45·5	0·00372	0·406	109
,, 60 ,,	15·6	4840	43·6	0·00323	0·358	111
,, 120 ,,	13·5	4180	37·1	0·00322	0·362	113
,, 180 ,,	11·1	3440	31·4	0·00322	0·352	110
,, 240 ,,	8·32	2520	23·0	0·00330	0·361	110
149° F. 30 ,,	9·97	3210	28·1	0·00311	0·355	114
,, 45 ,,	6·95	2200	18·9	0·00316	0·367	116

(464) Starch Conversion by β-Amylase.

The mechanism of starch conversion by β-amylase suggested by Ohlsson and illustrated in Fig. 115, is supported by a good deal of experimental evidence with purified barley amylase. Maltose is the only reducing product, and the conversion of soluble starch at p_H 4·5–7·0 comes to a very definite conclusion when 60 per cent. is converted to maltose. Blom, Bak and Braae[2] found that the conversion was limited to 53% at p_H 3·5. The products, β-60-dextrin and β-53-dextrin, according to these authors' nomenclature, are white powders and apparently represent the last two of a series of starch-like dextrins, both giving a blue-violet colour with iodine. The last is usually known by the name given to it by Baker (Section 127), α-amylodextrin, but numerous other names have been used for the same product, Table 202.

TABLE 202.—DEXTRINS PRODUCED BY β-AMYLASE

Name	Authors	$[\alpha]_D$	R maltose = 100	% starch converted to maltose
Erythrogranulose	Wijsman (1889)	—	about 1	—
α-amylodextrin ..	Baker (1902)	190°–195°	0·55–1·0	60–65
α-β-hexa-amylose ..	Ling and Nanji (1923) ..	193°	negligible	66·7
α-amylose ..	Klinkenberg (1932)	194°	negligible	—
Limit dextrin 1 ..	Syniewski (1925)	193·3°	1	65
α-amylodextrin ..	Freeman and Hopkins (1936)	188°	1	60–63
α-amylodextrin ..	Haworth *et al.* (1935)	200°	3 (hypoiodite)	—
-β60-dextrin ..	Blom *et al.* (1936)	185°–195°	0·5	60
β-53-dextrin ..	,, ,, ,, ...	185°–189°	0	53

The cessation of conversion at 60% was tentatively explained in Section 461 by the failure of β-amylase to overcome the resistance of an aggregated part of the starch, in which 12 links of a number of macromolecules are bound together in some way in which phosphate may possibly be concerned. This represents α-amylodextrin as a pre-existing fraction of starch, of different structure from the part readily converted to maltose. Malt amylase, free from maltase, acts rapidly on α-amylodextrin at 77° F., until 35–40% of the theoretical quantity of maltose is formed, with achroodextrin, but no glucose. The hydrolysis continues after this stage at a relatively slower rate, producing glucose almost exclusively (Bailey, Hopkins and Dolby[14]). α-amylodextrin is usually obtained from barley amylase, but the absence of α-amylase has, until recently, not been assured and the possibility of the existence of another liquefying enzyme hardly considered in experiments with β-amylase. Some doubt must consequently remain as to whether the further slow breakdown of α-amylodextrin, reported by several authors, was due to β-amylase.

Even after every care has been taken in the purification of enzyme preparations, it is only with great reserve that their homogeneity can be claimed, and the results of experiments with them are, consequently, never quite free from doubt.

Freeman and Hopkins[15] found that α- and β-amylases both readily and completely hydrolysed the amyloamylose component (about 20%) of starch and a portion of the erythroamylose component. Both enzymes degraded much the same fraction of starch, about 60%, but whereas the remainder, or α-amylodextrin, was almost if not entirely resistant to β-amylase, it could with difficulty be hydrolysed by α-amylase. When the breakdown had been commenced by α-amylase, it could be carried somewhat further by β-amylase, as if α-amylase held the key to further progress.

(465) Starch Conversion by α-Amylase.

There appears to be no sharp limit for conversions with α-amylase, but several authors have noticed that the hydrolysis was checked when the reducing power was represented by about **R** 33, became slower still at **R** 40, and almost ceased at **R** 47. It rapidly induces those changes that result in cessation of the colour reaction with iodine solution. The "transition point" from blue to red occurs when the reducing power of the conversion mixture corresponds with about **R** 14–15 and the "achroopoint," where colouration ceases, at about **R** 29–32. Conversions by α-amylase differ from those with β-amylase in that the dextrins produced have considerable reducing power and cannot be separated cleanly by alcohol fractionation. The maltose can, however, be fermented and its quantity estimated by determinations of reducing power before and after fermentation. Using a pure α-amylase preparation from the commercial bacterial amylase, " Superclastase," Blom, Bak and Braae[16] found that maltose does not appear until about 6–7% of the starch has been hydrolysed. The quantity of maltose in the products increased to about 23% when 40% of the starch was broken down. After this there was no further production of reducing dextrins. Maltose is the only crystallisable sugar produced by α-amylase, and the dextrins split off appear to consist of a mixture of comparatively short chain compounds. Myrbäck[17] separated 11 fractions from the " limit dextrin " produced by prolonged action on maize starch of Taka-amylase, which is substantially α-amylase, and found that they had molecular weights between 500 and 1,000, but more than 60% had a molecular weight of about 950. This closely corresponds with the suggestion in Fig. 115 that the original cleavage products consist of 6 glucose residues with a terminal reducing aldehyde group. These are apparently subsequently reduced to shorter chain compounds, tetra- and tri-saccharides with maltose. As the dextrins are removed, either from the end of the chains or, as alternatively suggested, by splitting them at some other points, more complex residues would also be left.

The shorter chain dextrins are characterised by increasing solubility in alcohol, lower optical activity and greater reducing power. Theoretical constants for various short chain dextrins are given in Table 203. These were calculated by Hanes from Freudenberg's[18] equation, based on a chain length of 30 units and $[\alpha]_D$ of 200° for starch, with $[\alpha]_D$ 138° for maltose, assuming that the terminal aldehyde group exerts its full reducing power. It may be noted that the reducing power of starch is considerably less than would be expected from 30 glucose links terminated by a free aldehyde group, from which it may be supposed that the union of molecular units occurs at these groups.

TABLE 203.—EXPECTED CONSTANTS OF SHORT CHAIN DEXTRINS (HANES)

Glucose units	3	4	5	6	7	8
R (maltose = 100) ..	68	51	41	35	30	26
$[\alpha]_D$	159	170	177	181	185	187
Molecular weight ..	504	666	828	990	1153	1314

There was very close agreement between the reducing power and maltose content found by Blom, Bak and Braae for 1-hour conversions with α-amylase at 77° F. and those of Baker and Hulton for a 12-hour conversion with malt amylase at 165° F. They were R 34·3, maltose 14·4% and R 35·2, maltose 14·8%, respectively. At 165° F. the β-amylase of malt diastase would be rapidly destroyed and the conversion would be substantially by α-amylase. It is permissible to assume that the unfermentable dextrins were the same, consisting largely of the six-unit reducing dextrin or "maltodextrin," as indicated in Fig. 115. Freeman and Hopkins[19] fractionated conversions that had only been carried to R 20·6 and R 29·6 and obtained major products with reducing powers of R 20 3 and R 23·0, which would apparently correspond with dextrins of greater chain length. The products given in Table 200 may be considered as representative of dextrins obtainable by α-amylase. They are not readily degraded by α-amylase, but easily converted to maltose by β-amylase and may thus be regarded as intermediates in the conversion of starch.

(466) Starch Conversions by Joint Action of α- and β-Amylases.

The account of the individual activities of α- and β-amylases emphasises the point that they act on starch simultaneously and not necessarily successively, but, when they are both present, either as a mixture of pure enzymes or in malt diastase preparations, that the α-amylase greatly facilitates the rapid production of maltose by producing a greater number of end groups in short chain dextrins for hydrolysis by β-amylase. It is necessary, however, to take into account the different thermo-stabilities of α- and β-amylase when considering the results of conversions by mixtures of the enzymes or by malt infusions. The optimum temperature range for the activity of β-amylase is some 20° F. lower than that of α-amylase and it is destroyed much more rapidly at higher temperatures (Section 127). The share taken by α-amylase in conversions, consequently, increases relatively to that of β-amylase as the temperature rises, and those with "restricted malt diastase" at about 150° or higher temperatures are substantially catalysed by α-amylase alone. The products at different temperatures must therefore vary according to the relative activities of the two enzymes at the particular temperature.

The conversion of starch can be carried almost to completion by high concentrations of malt amylase, that is by α- and β-amylases together, at p_H 4·6 and 77° F., but under ordinary conditions and below 140° F. it comes to a resting stage when the products of hydrolysis have a reducing power equivalent to that of 80% maltose. The dextrin in this product is the "stable dextrin" to which reference has already been made. The dextrin cannot be satisfactorily separated from the conversion mixture by alcohol precipitation, but it remains unattacked after fermentation of the maltose by brewery yeast. An 80% conversion mixture obtained with purified α- and β-amylases was found by Blom, Bak and Braae[2] to contain 73·7% of maltose and 26·3 of dextrin, which, according to these authors' nomenclature, would be called α-β-80-dextrin. It can be completely fermented by yeast in presence of malt amylase.

Examples of products isolated by different authors from starch conversions with malt amylase at about 122° F. are given in Table 204. Variations in the published constants of the dextrin fractions may possibly be due to differences in the degree of purification. This is known to be the case with one of the products in the Table, the constants of a first fractionation by Baker and Hulton being given to compare with the limit dextrin obtained from it by further purification and illustrate the point. Ling and Carter[20] used methyl alcohol to secure complete purification. It is possible that some of the differences may also be due to the presence or absence of amylophosphatase in the enzyme used, whereby two distinct dextrins may have been produced, as is suggested by the constants.

TABLE 204.—PRODUCTS OF STARCH CONVERSION WITH "UNRESTRICTED" MALT AMYLASE PREPARATIONS AT ABOUT 122° F.

Name	Authors	Conversion mixture		Fractionated dextrin	
		R	$[\alpha]_D$	R	$[\alpha]_D$
Stable dextrin	Brown and Heron (1879)	80·9	146·4	—	—
,, ,,	Brown and Millar (1899)	80	150	5·7	196
Polyhexosan	Ling and Carter (1938)	80	150	0	200
Limit dextrin	Baker and Hulton (1937)	80·3	147·1	5–10	185–190
First fraction (crude)	,, ,, ,, ,,	80·3	147·1	21	172
Stable dextrin	Ling and Nanji (1925)	80·8	147·5	14	185
Limit dextrin	Lintner and Kirschner (1923)	79·8	145·3	22·9	179
α-β-80-dextrin	Blom, Bak and Braae (1936)	79·1	147·0	22·9	175

(467) Mash Tun Conversion.

Great caution is necessary in applying the results of experiments with purified starch and amylase preparations to the very different conditions existing in the mash tun, where the concentrations of enzymes and substrate are greater and complications may be introduced by salts, maltose and proteins. The activities of the extracted and precipitated enzymes probably differ from those of the natural enzymes of malt, and some part of the latter is insoluble or only liberated by proteolysis. No satisfactory method for estimating the activities of the individual enzymes at various times or temperatures in the mash has been described, but it is probably justifiable to conclude that the experimental results may be applied on general lines. The balance between activation and destruction outlined in Vol. I; Section 123, must be borne in mind and the time factor taken into account. The temperature optima for the enzymes are considerably higher in the mash tun than *in vitro.* That of β-amylase, as indicated by the production of maltose, apparently lies between 140° and 148° F. and that of α-amylase between 158° and 170° F. Table 201 shows that β-amylase in dilute solutions is fairly stable at 140° F., but that its activity is reduced by about 40% in 30 minutes at 149° and probably inhibited much more rapidly at 158° F. It might, however, be noted that the destructive effect of temperature in laboratory experiments appears to vary considerably with different enzyme preparations. Ohlsson exposed malt extracts to 158° F. for 15 minutes with little loss of activity.

Swanson and Kruzic[21] used Ohlsson's methods to destroy α- or β-amylase in samples taken from a mash at various times and temperature levels in order to obtain an idea of the active quantity of the other enzyme at those stages. They no doubt destroyed some of the α-amylase along with the β-amylase before determining the activity of the former by a modification of Wohlgemuth's iodine process, but their results should be considered. These show that the maximum active quantity of α-amylase existed in the wort in the latter part of the 10 minutes' stand at 133° F., during which its extraction would have been facilitated by proteolysis. After this there was a rapid destruction of the enzymes as the temperature was raised in 12 minutes to 154° F. Inactivation continued, but at a slower rate, during the 20 minutes' stand at this temperature for saccharification. The quantity of α-amylase remaining active after 30 minutes at 160° F. was very small, but its destruction was never quite complete, even after the temperature had been raised to 183° F. It would appear that destruction of α-amylase commences in a mash below 150° F. and that its apparently increased activity at higher temperatures is due to the greater reaction velocity.

Two other factors operating in the mash have considerable influence on the activity of the enzymes. These are (1) the protective influence of peptones, maltose and other substances (Lüers and Lorinser[22]), which tends to prevent rapid enzyme destruction, and (2) the reversible inhibiting action of maltose, by which the enzyme activity is reduced as maltose accumulates

(Wohl and Glimm[23]). This inhibition is not due to destruction of the enzymes, the activities of which are restored by removal of the maltose. It may consequently be supposed that the production of a small quantity of erythrodextrin at later periods of sparging, when maltose is removed, is partly due to restoration to short-lived activity of any α-amylase that may have survived the high temperature under the protecting, but temporarily inhibiting action of maltose. Starch and dextrins may also appear in the last runnings through the physical effects of hot liquor on granules locked in a protein matrix in the steely tips of corns.

The active quantity of β-amylase was similarly found to rise to a maximum when the temperature reached 133° F. Destruction was rapid during the 12 minutes in which the temperature was raised to 154° F. and very little remained after 20 minutes at this temperature and none at 172° F.

It would appear that β-amylase is thermally inactivated to a considerable extent in mashes at between 140° and 150° F., but that some part of that extracted from the malt would remain active during the 20 or 30 minutes required for saccharification. The velocity of reactions catalysed by the still-remaining enzyme would be very high at 150°–155°, and it may be supposed that β-amylase produces some of the maltose directly from starch as well as from the " maltodextrin " produced by α-amylase, which would also be very active. It is, however, improbable that dextrins of the α-amylodextrin type can persist in wort, since any formed by β-amylase would be further hydrolysed by α-amylase and β-amylase together. There can be no doubt that a considerable proportion of the reducing dextrins and " maltodextrin " formed by α-amylase escape conversion to maltose by β-amylase, particularly at higher mashing temperatures, and contribute to the " apparent maltose " of wort. They also account for the high angle of worts in which they exist in quantity and reduce its fermentability. It is also probable that wort contains a small amount of the " limit dextrin " of Baker and Hulton, together with reducing dextrins of greater complexity than " maltodextrin " and some intermediate between the latter and maltose.

The production of glucose in the mash has also to be accounted for. A small quantity may be derived directly from the malt in which it existed among the ready formed sugars. Its presence to the extent of 10 or 18% of the solids in diastatic malt extracts and in smaller quantity in worts from lightly cured malts, mashed at low temperatures, is probably due to the activity of maltase. This enzyme is inactivated at high kiln temperatures (Section 129) and, even if present in the malt, its activity would be greatly restricted at the lowest infusion mash temperatures. There remains the possibility that α- and β-amylases acting together are able to produce a small quantity of glucose, possibly from α-amylodextrin as indicated in Section 199. An account was given in Vol. I, Section 144, of a viscosity-producing substance in worts from very undergrown malts. Piratzky and Wiecha[24] have more recently modified their view of its nature and state that worts from a 3-day malt may contain about 4 grams per litre of a complex polysaccharide to which the viscosity was due. This had properties resembling the α-amylan of O'Sullivan (Vol. I, Section 58) and was broken down by amylases in the mash to yield maltose and glucose. This substance could not be detected in well-modified malts and was probably broken down during the later stages of malting.

The results obtained in experimental enzymic conversions are not in conflict with the view that the molecular unit of starch is represented by a chain of 30 glucose residues, and that the dextrins consist of similar but shorter chains. The properties that result from the existence of aggregated or resistant parts of the chain may be expected to persist in the dextrins that include them. The fermentability in presence of malt amylase of " stable dextrin " and "maltodextrin " that are unfermentable in its absence account for the properties formerly attributed to " high type maltodextrins." Baker and Hulton's experiments on the fermentability of their " maltodextrin " referred to a few yeasts only, and the possibility remains that other species of *Saccharomyces* or such torulae as *Brettanomyces* can ferment it and explain some cases of conditioning during storage. The actual mechanism of secondary fermentation of this type, however, remains obscure. Failure to isolate and identify the dextrins of wort has made it inevitable that numerous hypotheses should have been advanced in attempts to explain their nature and properties. Baker and Hulton's conclusion in regard to the small number of dextrins produced from starch differs fundamentally from that of others who would only limit the number of possible fission products by their estimate of the complexity of the starch molecule, and their opinion as to whether hydrolysis could take place at random points in the starch chain or was restricted to certain linkages. The problem of the actual nature of starch conversion products in the wort still remains unsolved, and it is impossible to construct a balance sheet for its $[\alpha]_D$ and reducing power from values calculated from the constants of its probable constituents. In attempting this it is necessary to take into account the 4 or 5% of cane sugar already present in the malt.

(468) Pentosans.

According to Fink and Hartmann's analyses (Vol. I, Section 149) malt contains about 1% of soluble pentosans, and cytase, though of lower thermo-stability than β-amylase (Section 128), is possibly still active in an infusion mash and may hydrolyse hemicelluloses, of which malt contains 5–10%, mainly in the husks (Sections 55 and 56). The products of their hydrolysis are even less well known than those of starch, but they must include pentosans, pentoses, hexosans and hexoses, because hemicelluloses contain both pentose and hexose units in their molecules. It is seldom that any attempt is made to estimate the quantity of pentoses or pentosans in wort, but they may account for 2–4% of the total solids, and may be supposed to have some influence on the fulness and head-retention of beer, while other substances derived from the husk and probably related to them give flavours which are more or less harsh. A small quantity of tannin is also extracted from the husks and may have a somewhat astringent flavour, and increase the colour of wort and beer, through oxidation to phlobaphenes (Sections 164 and 165). Their reactions with proteins and association with haze production are dealt with in Sections 496 and 709.

THE NITROGEN OF WORT

(469) Wort Protein.

This term is frequently used to cover the whole of the nitrogenous constituents of wort, regardless of the fact that these consist mainly of protein degradation products with some soluble compounds not related to proteins. The quantity of true protein constitutes a relatively small proportion of the total nitrogen compounds, which altogether account for only 3–5% of the wort solids. The typical water-soluble, coagulable protein of malt, the albumin, is extracted in the mash and a small proportion is probably degraded to permanently soluble products, but the greater part is denatured and coagulated either in the mash tun or during copper boiling. The globulin of malt is soluble in dilute salt solutions, but the concentration of salts in the mash is probably too low to cause more than partial solution. Globulin is denatured by boiling, but not nearly so readily coagulated as albumin and, if present in its original form, is more likely to survive in boiled wort. The coagulation of proteins is never quite complete unless the p_H value of the medium is close to their isoelectric points. Since that of albumin is at about p_H 4·6, some may escape removal, even when boiled with hops, particularly in more alkaline worts. In any case, a slight quantity of protein or some closely related degradation product, probably combined as an adsorption complex with tannin, hop resin and silica, does survive copper boiling and can be precipitated by further intensive boiling. Hartong[25] has attributed chill haze to precipitation of dehydrated, colloidal particles of globulin, with adsorbed P_2O_5, silica and other anions and cations of salts, in combination with tannin, pectin and other substances (Section 709). It is, however, possible that the substance or substances concerned may be metaproteins rather than globulin in its original form.

(470) Protein Breakdown.

Malt contains representatives of all the groups of protein breakdown products described in Vol. I, Section 113. Proteinase and peptidases (Section 131) were concerned in their production, but the latter are inactivated at the kiln temperatures adopted for most English malts, though they may survive light curing. In the latter case it is doubtful whether they withstand the high temperature of the ordinary infusion mash, but they probably remain active up to 130° F. In view of the increasing proportion of amino-nitrogen in the total nitrogen of the simpler degradation products, it is possible to obtain an approximate idea of the degree of proteolysis in wort by determining the amino-index of the permanently soluble nitrogen, that is the formol-nitrogen of the boiled wort expressed as a percentage of the permanently soluble nitrogen. It is in this way possible to judge of the " qualitative " effects of proteolysis as distinct from its " quantitative " effect, as ascertained by determination of the permanently soluble nitrogen. An argument for or against the activity of peptidase based on the amino-index is not very secure, in view of the lack of information on the limit to which proteinase can degrade the proteins. The highest amino-indices for amino-acids, dipeptides and tripeptides are 100, 50 and 33·3 respectively, and a figure approaching these would appear to provide evidence of peptidase action, since proteinase does not produce such simple compounds.

An amino-index of 41·4 for the permanently soluble nitrogen of the cold water extract of the pale lager malt in Table 175, Section 439, suggests considerable peptidase action during malting, while the very small decrease at 104° and 122° F. indicates its continuance with rather reduced intensity in mashes made at these temperatures.* The considerable fall in amino-index at 140°

* The high percentage of amino-nitrogen in many of the Continental analyses, may be attributed to defects in the method of formol-nitrogen estimation adopted. The results are, however, parallel with those obtained by the method described in Volume III, though about 5% higher, and lead to similar conclusions.

and 158° suggests its restriction or inhibition. The corresponding figures for the formol and permanently soluble nitrogen produced during mashing bring this out more clearly. It seems impossible to attribute an amino-index of 37·6 and 36·2 to products of proteinase action alone, but 26·3 and 24·7 obtained at 140° and 158° may bear this interpretation. The lower figure means more complete inhibition of peptidases or less profound proteolysis by proteinase. The figures in Table 190, Section 449, showing the influence of p_H as well as of temperature, bear a very similar interpretation. At 104° F. and p_H 6·5 there was an actual increase in the amino-index of the permanently soluble nitrogen over that found at 32° F. and the same p_H value. This reaction is much more favourable to peptidase than proteinase (Section 131), which makes it appear probable that peptidase remained active in that particular malt and at a mashing temperature of 104° F. The *quantity* of formol nitrogen at any given temperature was, however, greater at lower p_H values, which suggests that the increase was due mainly to proteinase, as is confirmed by calculating the amino-index of the P.S.N. produced in the mash at 104°. At p_H 6·5 it was 42 and at p_H 4·6 it was 29. Corresponding figures at 140° F. are 22 and 28, which suggest restriction of proteinase activity at p_H 6·5 and the possibility that peptidase was not concerned in the proteolysis at that temperature.

The general conclusion from these and similar analyses, including those of Kelly, with a Californian malt (Table 188), is that peptidase, even if existing in the malt, is inactivated at the comparatively high temperatures of the infusion mash, but that it probably remains active in low temperature mashes, during a protein rest and in the mash used for manufacture of malt extract. In the latter case peptidase action may be considerable on account of the low drying temperatures to which the malts are subjected. In such cases it must modify the composition of the wort to a greater extent than is possible by the activation of proteinase alone.

(471) Fractionation of the Nitrogen of Wort.

The various properties of wort that depend on its nitrogen content are held to be associated with groups of nitrogenous substances, differing in the stage of protein degradation they represent, that is in their colloidal state or molecular magnitude and ability to diffuse through the cell walls of yeast, by which they become potential yeast nutrients. They have so far eluded all attempts at sharp fractionation, the proteins, metaproteins, proteoses, peptones and polypeptides grading imperceptibly one into another, so far as their properties are concerned. The difficulties of fractionation are greatly increased by the colloidal character of the more complex nitrogenous substances, and the consequent limitation of the terms used to distinguish fractions obtained by different methods of analysis must be borne in mind. Some of the proposed methods have, despite their restrictions, proved of use in estimating the proportion of the nitrogen of wort which might reasonably be supposed to be detrimental to the quality of beer or which might be useful for yeast nutrition, head formation or palate fulness. Since none of the methods so far proposed give sharply defined fractions, differing in their chemical constitution or properties, their value must depend on their reproducibility and usefulness for comparative purposes. The simple methods of analysis, giving the coagulable, permanently soluble and amino-nitogren provide, in most cases, all the information necessary, but examples have been given of the use of others in special circumstances.

Lundin[26] devised a method of nitrogen fractionation that is rather widely used and gives reproducible results, with three fractions that have a reasonably close approximation to groups of nitrogenous substances with characteristic properties. Separations are made by precipitation from the wort by tannin and phosphomolybdic acid.

Fraction A, precipitated by tannin, probably represents the high molecular nitrogen compounds and includes the nitrogen coagulated by boiling. It is held to give information in regard to the nitrogen compounds which, if not removed during boiling and cooling, are liable to lead to haze production in the beer.

Fraction B. Phosphomolybdic acid carries the precipitation further. The difference between the nitrogen precipitated by this reagent and by tannin is referred to as " fraction B " and is assumed to represent nitrogen compounds of medium molecular weight, including such protein digestion products as peptones and higher polypeptides.

Fraction C is the nitrogen not precipitated by phosphomolybdic acid and is derived by subtracting the phosphomolybdic nitrogen from the total nitrogen of the wort. C = Total N = (A + B). It includes the nitrogen of the lower polypeptides and amino-acids, and is an indication of the more readily assimilable yeast nutrients.

The analysis in Table 194, Section 451, provides an example of fractionation by these reagents. The wort with a specific gravity of 1068, represented in the first column, may be calculated to contain 0·125 gram nitrogen per 100 ml. The results of the fractionations are given in Table 205.

TABLE 205.—FRACTIONATION OF WORT NITROGEN

	Nitrogen			Nitrogen	
	gr. 100 ml.	% wort N.		gr. 100 ml.	% wort N.
Fraction A ..	0·037	29·5	Coagulable ..	0·0113	9·05
Fraction B ..	0·016	12·9	Perm. Soluble ..	0·1136	90·85
Fraction C ..	0·072	57·6	Formol	0·0467	37·4

True proteins and some of their most complex derivatives are sometimes salted out by saturating the wort with magnesium sulphate, after acidification to p_H 1–2 with sulphuric acid, and attempts are sometimes made to determine the quantity of " colloidal nitrogen " by adsorption on active carbon. (Table 198, Section 459.) This might be supposed to represent nitrogenous substances adsorbable by yeast, etc., but the results cannot be regarded as very conclusive, since a small quantity of simple nitrogen compounds is also adsorbed. Zinc sulphate and phosphotungstic acid were used by Brown and in the Guinness Researches, and have since been employed by many authors, who generally attribute the fraction salted out by zinc sulphate to "albumose" nitrogen and that afterwards precipitated by phosphotungstic acid to " peptone" nitrogen. The difference between the total nitrogen and that included in these two groups, together with the formol, titratable and coagulable nitrogen, would then be attributed to " polypeptide nitrogen." Useful as these processes may be for comparative purposes, when some idea of the relative proportions of complex and simpler compounds is required, they are of no actual value for differentiating groups of allied compounds. The extent of precipitation depends on the relative concentrations of the solutions and precipitant.

Attempts are also sometimes made to estimate the quantities of wort nitrogen combined in different ways, as ammonia-nitrogen, amino-nitrogen, amide-nitrogen, and peptide-nitrogen, for example. Nitrogen given off as ammonia by warming the wort with a small quantity of lime *in vacuo* is regarded as ammonia-nitrogen. The amino-nitrogen may be estimated by the Van Slyke or Sörensen method. The amide- and peptide-nitrogen can be estimated by measuring the increase in ammonia or amino-nitrogen caused by hydrolysis of the wort by acid. The results of this kind of analysis have not yet proved of much value, as they can only give a very approximate idea of the nature of the nitrogenous constituents of wort and leave a large proportion of the nitrogen unaccounted for. The latter includes practically all the colloidal nitrogen that can be adsorbed on active carbon. The quantity so removed varied between 14 and 34% of the total nitrogen of bright boiled worts in different experiments by Schryver and Thomas.[27] An average value for these nitrogen fractions in a number of analyses of boiled worts from English malts are given in Table 206. A proportion of the undetermined nitrogen is due to nitrogen of amino and peptide compounds, not determined by the Van Slyke and hydrolytic methods employed. On an average, about one-third of the nitrogen of the determined groups was found to be removed during fermentation, the largest difference before and after fermentation being in the peptide group, which was originally present in largest quantity. The undetermined nitrogen was apparently not assimilated, though a proportion might be removed by precipitation or adsorption on the yeast.

TABLE 206.—AVERAGE NITROGEN DISTRIBUTION IN ANALYSES OF 12 WORTS FROM ENGLISH MALTS (SCHRYVER AND THOMAS)

Nitrogen groups	% of P.S.N.
Ammonia-nitrogen	3·1
Amino-nitrogen (Van Slyke)	17·8
Amide-nitrogen	8·1
Peptide-nitrogen	28·2
Undetermined nitrogen	42 8

(472) Classification of Wort Proteins on the Basis of Amino-index.

The usually accepted definitions of proteose and peptone, based on the study of animal proteins, is that they are intermediate fractions of protein breakdown, which can be separated by different degrees of saturation with magnesium sulphate. The corresponding intermediates derived from plant proteins are not clearly differentiated in this manner. Bishop suggests a re-definition on what is considered a sounder basis, that of the amino-index. This permits of a tentative nomenclature of groups, based on the percentage of amino-nitrogen on the total nitrogen.

(*a*) Proteins, with	2–3·5%	free amino-nitrogen	
(*b*) Proteoses and peptones	6–18%	,,	,,
(*c*) Polypeptides	20–30%	,,	,,
(*d*) Simple peptides	more than 30%	,,	,,
(*e*) Free amino-acids and amides	up to 100%	,,	,,

The latest investigations carried out at the Institute of Brewing Research Laboratories, some of the results of which have been privately communicated to the author by Dr. L. R. Bishop, indicate that phospho-tungstic acid, added in progressively increasing quantities, serves as a means of fine fractionation of the nitrogen compounds of wort, and for their quantitative estimation. Tentative conclusions, reached from analyses of worts and beers by this method, are that the nitrogen compounds of the worts examined fell mainly into the following categories, in order of complexity as measured by their amino-indices, Table 207.

TABLE 207.—AMOUNT OF FREE AMINO-N AND AMIDE-N OF WORT NITROGEN COMPOUNDS (L. R. BISHOP)

	% of total nitrogen	Approximate composition, % of N	
		Amino-N	Amide-N
Proteins and Metaproteins	5–10	3·5	10
Proteoses and Peptones	25–30	12	8
Polypeptides	25–30	28	10
Free Amino-acids and simple Peptide compounds	30–40	40	—

(473) Functions and Properties of Wort Nitrogen.

About 25 to 30% of the wort nitrogen has been found by such methods of analysis to exist in the form of protein derivatives of medium complexity, included in the rather indefinite groups of proteoses and peptones. These retain many of the characteristics of fully built-up proteins, are of colloidal dimensions, but are not coagulated when the wort is boiled. They remain in dispersion in hot wort, partly combined with tannins from the malt and hops, but a large proportion is precipitated from the wort on cooling and during fermentation. Any of the so-called protein-tannin or peptone-tannin which escapes precipitation during brewing or storage of the beer is liable to come out of solution and form a haze. (Section 709.) Another proportion of the proteoses and peptones remains permanently in the beer. Its functions are organoleptic and depend largely on the colloidal state of the substances in question, which contribute to the flavour, palate fulness and foaming properties of the beer. In contributing to these properties they are probably more important than the unfermentable carbohydrates, although their quantity is smaller. Some 50–60% of the soluble nitrogen of wort is capable of being utilised by yeast, but the amount assimilated during fermentation does not usually exceed 40–50%. (Section 439.) This exists in the form of the simplest protein degradation products which are capable of diffusing through the yeast cell walls. The amino-acids and dipeptides are among the nitrogenous substances known to be utilised by yeast as a source of nitrogenous nutriment. (Section 678.) Wort also contains many substances which would be included in the group of polypeptides, ranging in molecular magnitude from that of the simple diffusible substances until they merge with others of colloidal dimensions and participate in the properties characteristic of the latter.

(474) Identified Nitrogenous Constituents of Wort.

Very few nitrogenous substances have been separated from wort in a form in which they could be definitely identified. Brown[28] mentions asparagine, with a little leucine, tyrosine and allantoin among the amides and amino-acids extracted in a crystalline form from cold water malt extract in the Guinness Researches. Tryptophan and β-indol-α-amino-propionic acid and hordenin have also been identified. Asparagine occurs in the greatest quantity, but the nitrogen in these forms is small, being included in the 8·5% of the total permanently soluble nitrogen, classed by Brown as amino- and amide-nitrogen. The organic bases betaine and choline were isolated from the portion of the nitrogenous bodies precipitable by phosphotungstic acid, and accounted for about 4% of the nitrogen of the cold water malt extract. Miskovsky[29] identified and estimated the following in Pilsener beer, containing 36·96 gr. of dry substance and 0·308 gr. of nitrogen or about 1·925 protein per litre: Choline 0·078, Betaine 0·033, Arginine 0·005 and Histidine 0·002 gr. per litre. The amino-acids and asparagine are no doubt available as yeast nutrients, but the organic bases can hardly have any significance in the small quantity in which they occur.

(475) Summary.

The very complex structure of starch and the nature of the dextrins of wort derived from it by enzymic breakdown may be summed up in a homely simile, based on a suggestion by Hopkins, which will also afford a working hypothesis of its conversion. The colloidal particles may be represented by a faggot of sticks and a pile of these would be a granule. The complete pile must be demolished before conversion, and its constituent faggots separated, an operation that is partly performed during malting. The faggots would then be untied to liberate the sticks, a process which may roughly correspond with liquefaction. The individual sticks, possibly of different lengths, might then represent the molecular units of starch, while their conversion products would be the varying sized pieces of kindling wood into which they are chopped. The conversion products on further enzymic breakdown provide the heat that causes rise of temperature during fermentation.

If amylophosphatase is regarded as the implement by which the sticks in the original faggot are liberated, α- and β amylase would be the tools by which they are cut into smaller pieces. These are supposed to operate differently, β-amylase can only be used to chop off small pieces (maltose) from one end of the sticks, while the other can saw them in large sections (dextrin) from which short lengths may afterwards be chopped. It may also be suggested that one end of the faggot is not completely untied in the preliminary operation, so that the chopper, β-amylase, can only be used on a portion of the sticks, meeting opposition when the bound end is reached. The latter would then represent α-amylodextrin (Vol. I, Section 127), which can, however, be sawed in pieces by α-amylase.

The complication of starch conversion involved in the varying physical properties of the colloidal particles may be visualised by imagining that some of the sticks of some of the faggots are more tightly bound together than those of others. This would represent the difference in colloidal aggregation of amylopectin and amylose, and suggests that the latter may be much more readily and completely broken down to maltose. The invariable production of a " limit dextrin " in enzymic breakdown of starch may be represented by knots in some of the sticks or tightly bound ends of the faggots, which absolutely resist both the saw and the chopper. These, like their prototypes in wood, may be supposed to vary in size and position.

The imaginary reconstruction of the starch particles in terms of sticks, 30 feet in length, say, to represent the 30 glucose units, and the further implication that the saw and chopper are only able to cut the sticks at prescribed places into definite lengths of 6 feet and 2 feet lead to a working hypothesis of the nature of the starch conversion products of wort. Removal of six-foot lengths from the ends or middle of sticks would represent the production of the reducing dextrin that has been called " malto-dextrin," and would leave various other lengths as reducing dextrins of different chain length. Immediately these are sawed off, two-foot lengths of maltose could be chopped from any of them and at the same time would be being chopped from the end of unsawn sticks. If the workman possessed a finer tool, maltase, he might also be cutting some single-foot lengths of glucose. It follows that the fragments representing the starch conversion products in wort would be mainly two feet in length, maltose, some six-foot lengths, maltodextrin, and various odd lengths, greater or less than the latter, also reducing dextrins, with knots of limit dextrins and perhaps some glucose. Shorter pieces would predominate if the sawing and chopping were allowed to go on for a long time, and longer pieces if they were stopped more quickly. This represents the effect of heat on the enzymes. The higher the mashing temperature, the more rapidly they are arrested in their work, the chopper being stopped before the saw.

This picture of the enzymic hydrolysis of starch suggests that wort contains a large variety of its conversion products, a view that is widely held but cannot, at present, be decided by analytical methods. The alternative of studying the conversion of pure starch by diastase preparations can only be applied inferentially, and has led to rather conflicting results. Baker and Hulton,[30] however, came to the conclusion that the number of starch-conversion products in wort was probably very limited. The most important were maltose and two unfermentable substances, which they described as " maltodextrin " and " stable dextrin." These two would constitute the bulk of the carbohydrates in beer at racking. The former had a reducing power of about one-third that of maltose, and would account for some of the " apparent maltose " in brewery worts. It was not found in starch conversions carried out at temperatures below 122° F., but was found in conversions made at 149° and, in greater quantity, at 165°. It thus appeared to be an intermediate product of starch breakdown, owing its existence at higher temperatures to restriction of β-amylase which, at 122° and below, hydrolysed it completely to maltose. It is a definite compound, which can be isolated in a pure state. The name applied to it has no structural significance and does not imply acceptance of Brown and Morris' discredited " maltodextrin " theory. The " stable dextrin " was believed to be the residual part of the starch molecule, which resisted the attack of α- and β-amylase at all temperatures. Though Baker and Hulton were only able to isolate these two unfermentable products and found no fermentable sugar, other than maltose, they do not deny the possibility that others are present, and recorded the occurrence of a low molecular substance, with a specific rotation approaching that of maltose, and a reducing power of R 40–42. Whether the number of starch-conversion products in wort be great or small, they must be accompanied by analogous substances, produced from such malt constituents as hemicellulose, by cane sugar, which exists among the ready-formed soluble carbohydrates of malt, and probably by glucose and fructose.

The protein constituents of wort may be visualised in a somewhat similar manner, but it is better to represent the protein as a building constructed of different coloured bricks, including many of each colour. (Vol. I, Section 111.) These coloured bricks are the amino-acids and a large number of different amino-acids form part of each protein of barley. Two, or perhaps more, tools are available to start demolition of the building during malting; these represent a proteinase and, possibly, two peptidases. The former is the rougher tool that breaks the structure into its main component parts, representing proteoses and peptones, according to their size. Then the second tool, or peptidases, separates these into smaller fragments consisting of comparatively few bricks, polypeptides, of four to two bricks, tetrapeptides to dipeptides, and even into single bricks or amino-acids. This tool is, however, easily blunted and can no longer operate if it becomes too hot by rapid working, as may happen at high kiln or mashing temperatures. This picture of protein structure as a building of many coloured bricks makes it clear that the fragments constituting wort proteins may consist of many more different substances than the corresponding starch derivatives. The chance that the finer tool or peptidase may not be sharp enough to work in the mash suggests that work of demolition during malting, modification, must be carried further to produce sufficient single bricks for yeast nutrition if the mashing temperature is high. The preliminary breakdown during malting need not be carried so far if both tools are going to be allowed to work in a low temperature mash, and must be somewhat restricted if it is desired to retain a considerable quantity of the larger pieces of several bricks to give palate fulness and foaming properties to the beer.

The resulting protein derivatives in wort may be classed as :

(*a*) Simple compounds, assimilable by yeast.

(*b*) Complex compounds, not assimilable by yeast. These remain in the beer to contribute to head retention, fulness and haze production.

Small quantities of a few organic bases have also been detected.

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WORT BOILING AND COOLING

WORT BOILING AND COOLING

CHAPTER XXVIII

WORT BOILING

PLANT AND PROCESSES

(476) Objects of Wort Boiling.

The objects of the boiling process may be summarised as follows :—

(1) Evaporation of excess water.
(2) Destruction of enzymes.
(3) Sterilisation.
(4) Extraction of flavouring, preservative and other substances from the hops, involving changes in their chemical composition or colloidal state.
(5) Coagulation of proteins.
(6) Promotion of reactions between proteins and hop extractives.
(7) Completion of salt reactions.
(8) Caramelisation of sugars in dark worts.
(9) Cooking of unfermentable extract.

Evaporation of excess water is necessary for stronger beers, because a greater quantity of sparge liquor is generally required to secure the maximum extract from malt than the desired final gravity will permit. The motion associated with ebullition is beneficial in all cases. Destruction of enzymes, if not already effected by a temperature of 170°–180° F. in the mash tun or underback, is necessary to stabilise the composition of the wort by preventing further uncontrolled breakdown of carbohydrates by α-amylase, the only enzyme likely to retain any activity at this stage. Insistence on sterilisation may seem a counsel of perfection, in view of the chances of infection between the copper and fermenting vessel, but it can hardly fail to be attained and provides the foundation for the care taken to protect the wort from contamination. Extraction of the hops and removal of coagulable proteins are essential to the flavour and stability of beer. The chemical and physical changes involved in these processes are still only incompletely known, but it is clear that they are modified in ways that influence the character of beer by differences in plant, methods, time, vigour of boiling and various factors related to these. The stabilisation of hydrogen ion concentration, associated with salt reactions, is of importance in this connection, while the changes of flavour produced by caramelisation of sugars and increase of the digestibility of unfermentable extract by cooking are of great significance.

(477) Coppers.

In lager breweries, the wort copper or kettle is almost invariably in the same room as the mash and lauter tuns, situated at such a level that wort flows directly from the latter into it. The coppers of British top fermentation breweries are generally completely separated from the mash room, and it is usual to pump the wort from an underback, into which it runs from the mash tun. The underback may function simply as a well for the pumps delivering to the copper, but more frequently it is a cast iron vessel of sufficient size to take the whole or a considerable proportion of the wort from the mash tun and fitted with steam coils to raise the temperature of the wort to 180° F. and prevent further enzymic action. In the majority of lager breweries and some ale breweries, the coppers are of sufficient size to take the entire brew, but the practice of making parti-gyles of top fermentation beers makes it more convenient to have two or more smaller coppers for each mash. Many pale ale brewers restrict the size of coppers to between 60 and 150 barrels, according to the old Burton practice with brews several times this length, as these are believed to produce a smaller increase of colour than larger vessels.

The coppers of lager breweries are almost always surmounted by a dome and chimney to conduct the steam from the brewhouse, the system being, in many cases, extended by condensing the steam for mashing liquor or using it to heat warm liquor from the wort refrigerators. Domed coppers have an advantage over open for delicately flavoured beers, in that they reduce the contact with air and thereby tend to give paler beers, with a softer flavour. Domes of copper or stainless steel are less frequently fitted in ale breweries and the larger proportion of hops used tends to make the condensation of steam for mashing purposes more troublesome than economical, but it is satisfactorily carried out in some breweries and claimed to retain part of the hop aroma. Domed coppers, surmounted by a pan into which worts are run and heated by the boiling wort before they are let down into the copper as a second charge, are characteristic of London practice. A model of a copper of this type, fitted with an automatic stoking arrangement, is shown in Fig. 116.

Many coppers are fitted with fountains or open topped domes, over which the wort circulates when the dome is lowered to the level of the wort. These find many advocates, but others consider that a fountain offers no advantages, except with coppers that show an unusual tendency to boil over. A type designed to work with a central heater is shown in Fig. 117. The boiling wort discharged from the underside of the cowl beats down the fob and, in most cases, eliminates the danger of boiling over. Propellers are sometimes fitted in coppers to keep the hops in motion and prevent scorching. They take the place of chains, formerly used for the same purpose.

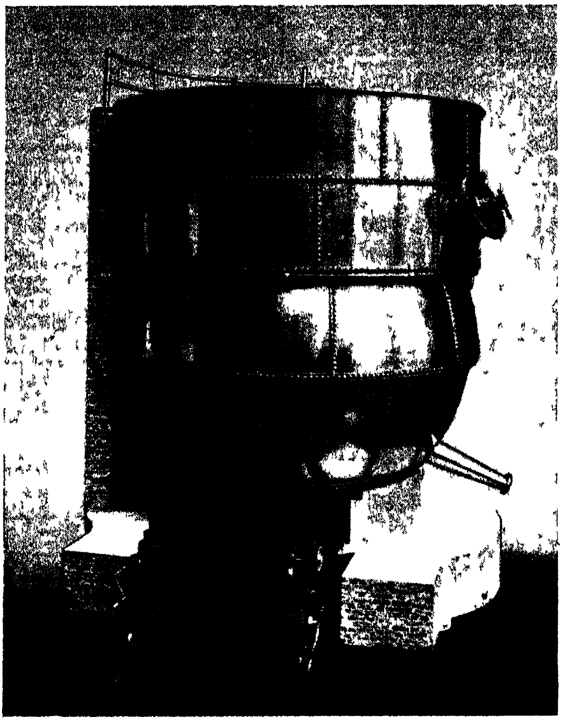

Fig. 116

MODEL OF COPPER SURMOUNTED BY WORT PAN
(TRUMAN, HANBURY, BUXTON AND CO., LTD., LONDON)

(478) Pressure Boiling.

Many brewers claim that a fuller flavoured beer is obtained by boiling the worts under a small pressure, such as is obtained by closing the copper and fitting it with a pressure valve, arranged to vent at between 1½ and 3 lb. The increased pressure raises the boiling point of the wort, as may be gathered from the figures in Table 208 for the boiling point of water under different pressures. A pressure copper is, therefore, of particular service in breweries situated at high altitudes, where the temperature of the boiling wort in an open copper may be considerably below 212° F. At Johannesburg, 5,764 ft. above sea level, an increase of wort temperature from 204° to 218° F. is recorded.

TABLE 208.—TEMPERATURE OF SATURATED STEAM AT VARIOUS PRESSURES

Lb. per square inch		Temperature F. °
Gauge pressure	Above vacuum	
0	14·7	212
0·3	15	213·1
1·3	16	216·3
2·3	17	219·6
3·3	18	222·4
4·3	19	225·3
5·3	20	228·0
15·3	30	250·4
30·3	45	274·4
45·3	60	292·7
60·3	75	307·5
75·3	90	320·2
90·3	105	331·3

As a result of the higher temperature of the wort in pressure coppers, the coagulation of proteins is accelerated and the break improved, sometimes causing a reduction of extract by 0·05–0·1%.

A certain amount of caramelisation may occur and be of advantage with dark beers. On the other hand, increase of colour due to oxidation is minimised and the extra colour produced is slight, if any, under low pressures, but an open copper is generally preferred for pale ales. Pressure boiling increases extraction from the hops and may be detrimental to the flavour of the beer if continued for too lengthy a period. On the other hand, the greater extraction permits of 5 or 10% economy in the quantity of hops used and, with comparatively low pressures and the reduced boiling times, the flavour of the beer should not be detrimentally affected, while the hop aroma may be improved, providing good quality hops are used. The evaporation is materially reduced, it may be 2 or 3% per hour, but this need not be a detriment and, in general, copper boiling under a pressure of 1½ to 2 lb. for 1 or 1½ hour is of advantage with the lighter running beers. It is economical in steam, on account of the reduced boiling time, and the super-heating of the wort is usually an advantage. Fig. 64 shows part of the copper stage of a large English brewery, with coppers of this description, designed to boil, when desired, under a pressure of 1½ lb.

(479) Fire and Steam Boiling.

Many brewers still adhere to fire-heated coppers. Others have adopted oil or gas burners, but steam boiling has advantages on the score of economy and convenience. The figures in Table 209 are from actual brewery tests and show an efficiency of 67% for steam applied in a central heater and 45% for a well set fire copper, Welsh steam coal being used in both cases.

TABLE 209.—COMPARATIVE EFFICIENCY OF STEAM- AND FIRE-BOILED COPPERS

	Fire	Steam	Theoretical
Coal for boiling 350 barrels wort, cwt. ..	30·8	20·6	13·85
Coal for boiling 1 barrel wort, lb. ..	9·85	6·6	4·43

The economy is actually greater than is here represented, because cheaper grades of fuel can be used in steam boilers than under a copper, with comparatively small loss of efficiency. Despite this economy, many brewers of repute still boil their coppers by direct fire. This may, in part, be due to conservatism or a very natural fear that the character of the beer might be altered by a change in boiling conditions. The usual claim in favour of fire is that a more brilliant, better flavoured and more stable beer is obtained, on account of better break in the wort and slight caramelisation. This has been attributed to the constant change in the position of maximum heat concentration and the very high temperature at this point. 377° F. has been recorded on the outside of the copper, just over the furnace bridge, with 257°–266° F. on the inside of the plate, compared with 239° on the wort side at the middle of the bottom of a jacketed copper boiled by steam at 60 lb. pressure (Field[1]).

The setting of a fire copper is designed to give a good central boil, with the greatest possible efficiency and avoidance of damage to the copper plates by excessive velocity of the flue gases. The heated surface is generally greater than with steam jackets or coils which, with the greater temperature difference, favourably influences circulation of the wort. In this respect, jackets and coils are probably inferior to coal furnaces. Rotating steam coils give good results, but the necessary joints present difficulties. Central steam heaters are very efficient in promoting circulation. They have a large heating surface in relation to their size and are made to stand considerable steam pressures. 60 lb. per square inch is not unusual, the temperature of the steam then being 307° F., but they should boil efficiently with 30–40 lb. pressure. Several designs are available; that shown in Fig. 117 consists of a copper drum, through which brass tubes pass from top to bottom. It is mounted on legs, one of which may be used as a steam inlet and another as exhaust. Another type consists of a cylinder open at the base, containing the heating elements and surmounted by rotating arms. The boiling wort rises through the cylinder and, setting the arms in rotation, is sprayed over the surface of the wort. Low pressure exhaust steam cannot be used for boiling, but high pressure steam has greatly reduced, if not extinguished, the advantage possessed by fire boiling in the early days of steam-heated coppers. The latter are now generally preferred, with steam pressures not less than 35–40 lb. per square inch.

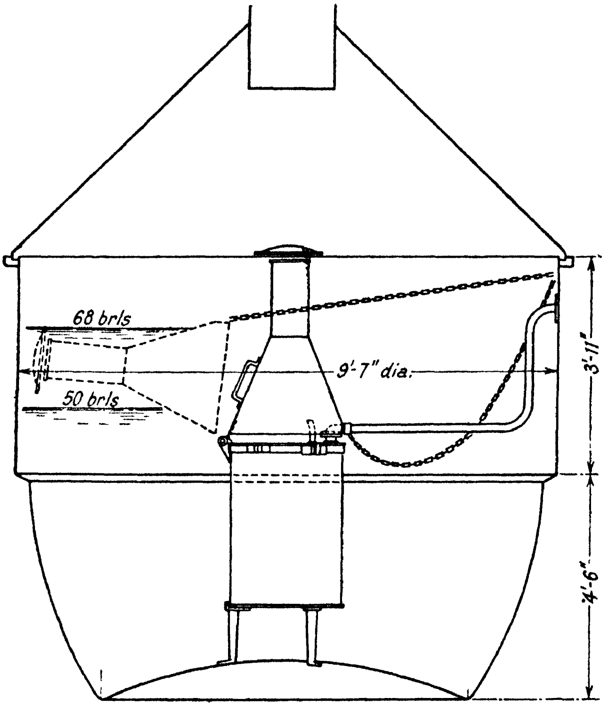

FIG. 117

COPPER WITH CENTRAL HEATER AND FOUNTAIN

(480) Filling and Casting Coppers.

Particular care must be taken with fire coppers to prevent damage to the plates when the wort is running in or after the copper is cast. The fire is not made up until the copper is "safe" or the wort has well covered the flue level, the temperature of the wort being then raised to 180° F., to arrest enzymic action, and to boiling as the last worts are running in. A similar precaution is taken by drawing the furnace before casting, or running out the worts. This not only prevents damage to the plates, but also burning of the wort left adhering to the copper. In order to assist in this, it is advisable to spray the surface with cold water as soon as the copper is empty, which can be done very conveniently by means of a hinged pipe with a rotating nozzle or small sparge arm. This prevents hardening of saccharine matter and facilitates cleaning.

The composition of the earlier and later runnings from the mash tun varies so widely (Section 450) that they behave differently if boiled separately. This difference is particularly marked in the character of the break, which may be very defective in weak worts if the sparge liquor is of alkaline composition. It is consequently desirable, when more than one copper is required for a brew, that the first and subsequent worts should be apportioned to them equally or in proportion to their sizes. In the case of parti-gyles, the relative proportions of strong and weaker worts must be varied in accordance with the gravities required, but some of each wort should be in each copper.

(481) *Addition of Hops.*

The required quantity of hops is usually weighed in the hop store and brought to the copper side while the wort is running in. They may all be added at once, shortly before the commencement of boiling, or in two or three portions at different times. Thus, one half may be added at the start, with one quarter after boiling for half an hour or an hour, and one quarter half an hour before turning out. This process is somewhat wasteful, in that all the useful constituents may not be extracted from the last portion, but it is considered in some breweries, though not in all, to give a better flavoured beer on account of the reduced loss of volatile oils.

According to Chapman, about 80% of the oils is volatilised with the steam when hops are boiled for half an hour with water, the remaining 20% being converted to slightly soluble resins. These have no antiseptic properties but retain some of the original flavour of the oils which is communicated to the wort.

If the worts are collected in more than one copper at different gravities, the hops are divided in proportion with the lengths and gravities, allowance being made for different types of beer in parti-gyles. The period at which the hops are added and the hop rate also influence the quantity of protein coagulated. For example, Kolbach and Wilharm[2] found 0·89 mgm. of coagulable nitrogen still remaining in 100 ml. of boiled wort to which 4 lb. per barrel of hops were added at the commencement of boil, compared with 1·69 mgm. when two-thirds of the hops were held back until a quarter of an hour before turning out.

(482) Hop Rate.

The quantity of hops used varies greatly according to the type of beer brewed, its gravity, mashing liquor composition, the quality of the hops, the season of the year and local taste. It is calculated either on the quantity of other materials used in the brew or on the basis of a determined weight per barrel or hectolitre of boiled wort or beer, e.g., as pounds per quarter of materials or pounds per barrel of beer. Examples of hop rates for various types of beer are given in Table 210, but there can be no fixed rule, as the quantity must be increased with old hops or in warmer weather, particularly as the latter occurs when the flavour and preservative properties of the hops have depreciated by 9 months' storage. An increase of from 10 to

TABLE 210.—HOP RATES

Type of Beer		lb. Hops per quarter malt, etc.	lb. Hops per barrel beer
Pale ale	1055	8¼–10½	1¾– 2¼
Pale ale	1048	7 – 8	1¼– 1½
Light bitter beer	1040	6½– 8	1 – 1¼
Mild ale	1040	5½– 7	⅞– 1⅛
Strong ale	1070	7½–12	2 – 3¼
Stout	1040	6½– 9½	1 – 1½
Stout	1055	7½–10	1½– 2
Pale lager	1040	2½– 5	½ – ¾
Pale lager	1048	3¼– 5¼	⅔ – 1¼
Dark lager	1048	2 – 3¼	½ – ⅔

1 lb. per barrel = 277·7 grams per hectolitre.
300 grams per hl. = 1·08 lb. per barrel.
1 lb. per barrel = 0·717 lb. per American Bbl.

25% in the winter hop rate may be desirable in the summer. New hops are rarely used before the end of December or early January, and then usually, at first, in relatively small proportion with the older hops, the quantity of new season's hops being gradually increased until the older are discontinued in the spring or early summer. The results of gravimetric analysis provide a good guide as to the relative quantities of different hops required to give consistent flavour and stability, since both flavour and preservative power are approximately measured by humulon and resin percentages. Less hops are required with a carbonate liquor than with a soft water or liquor in which the gypsum-carbonate ratio is well balanced, while a hard gypseous liquor permits the use of a larger proportion of strongly flavoured hops. With the same hops, carbonate liquors give more bitterness, but of a rough and harsh character. Soft waters give a softer beer, with a more delicate hop bitter, and wort from gypseous liquors can be hopped with a greater proportion of such strongly flavoured hops as most of the American varieties.

(483) Kind and Quality of Hops.

The varieties of hops used in any brewery must necessarily be influenced largely by those grown in the country or which can be economically imported. The choice is dictated by personal preference in respect of flavour or the likes and dislikes of customers, together with considerations in regard to preservative value. Preferred flavour often means that to which customers are accustomed or that of hops from the most accessible district. Even when the choice is wide, these preferences may be noted. Thus, there is a greater tendency to prefer Worcester or Hereford hops in the Midlands of England than in Southern districts, where Kent hops would be selected. The difference in the character of these hops is not nearly so great as that between either of them and most Continental or American hops, neither of which would, if used alone, generally be appreciated in English beers. On the other hand, the English hops and still less the Oregon Clusters find little favour for Continental lager beer, in which the various types of Continental hops are used. A blend of several types is almost always employed in English beers, the blends varying with the composition of the liquor and in the different kinds of beer at each brewery. Selection of the most suitable types is a most important matter in every brewery. It is based on the flavour detected on hand examination and demands very considerable experience and skill.

The Fuggle's is the most widely grown and extensively used of English hops and may be considered the most suitable type for mild ale copper use. Several growths of the same variety may be selected and blended in the copper to ensure regularity of flavour or a proportion of the so-called Golding Varieties may be used with the Fuggle's. This hop is also used in pale ales and bitter beers, but the more delicate flavour of Goldings may decide their selection, generally in blends with Fuggle's hops. Among the Continental hops used in lager beers, the German Hallertaus may be taken as corresponding with the Fuggle's, while the Saaz hops of Bohemia take pride of place as the most delicate. The Auscha and Styrian, with German Tettnang and Spalt hops, are also widely used for delicately flavoured beers. An increasing quantity of Hallertaus may be blended with them as a stronger flavour is required. Though variety, with which a characteristic flavour is generally associated, is an important factor in selection, it is far from all, as there are many grades of quality and differences of flavour due to locality or growth conditions. The samples must consequently be individually examined and the selected hops blended in the way that seems best suited to their individual characters.

(484) Hop Economisers.

Several methods have been proposed and are used in some breweries to effect an economy in the quantity of hops required or to make better use of their valuable constituents. In some cases, the hops are infused in hot liquor previous to use. This may open their cones and facilitate extraction when they are added to the wort, but a large proportion of the aromatic substances is lost by evaporation when the liquid is added to the copper. A better plan is to allow the hops to soak in cold water for 24 hours in a cylinder, provided with a perforated false bottom. By this means a very aromatic extract is obtained and can be added to the copper when boiling is completed, the extracted hops being added in the usual way. Machines are made by which the cones are partially broken and then passed over screens to sieve out the lupulin, which is added to the copper, shortly before turning out. The broken cones are added in the usual way, but the strigs are separated and rejected. This method may appear to offer advantages, but the machines are somewhat complicated and liable to become clogged, and filtration of the wort is also made more difficult by breaking the cones. In addition, constituents of the lupulin may not be allowed sufficient time for complete extraction or the chemical changes, which practice suggests is desirable. Though economies of 10% may be made in the quantity of hops required for flavouring purposes, these methods are liable to be accompanied by reduction in the quality of the beer or to introduce difficulties that counterbalance the saving.

(485.) Hop Back or Strainer.

Removal of spent hops is effected by passing the boiled wort through a hop back or strainer. The former is a cylindrical or rectangular iron or copper vessel, fitted with a false bottom.

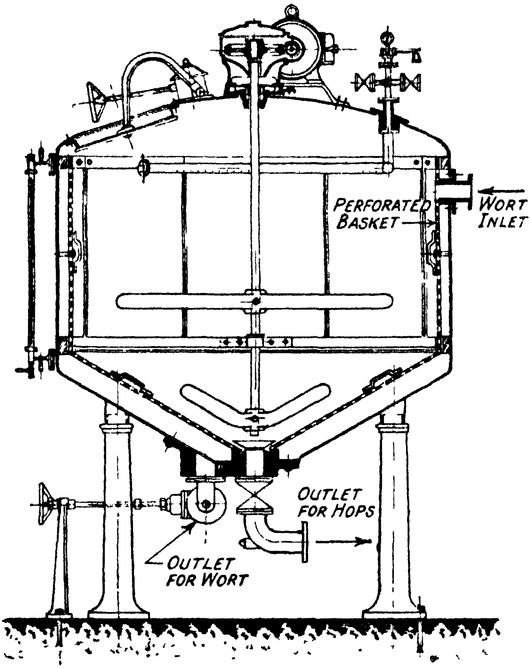

FIG. 118

HOP MONTEJUS

It should be of sufficient size to take the whole of the wort from a copper, but its cross section area should be limited so that the bed of hops is not less than 18 inches or 2 feet thick. In many cases the hop back is far too large for the size of the brew or quantity of hops used, and does not fulfil its duty as a filter capable of delivering bright worts. In such cases the back may be divided by transverse partitions into smaller sections or an inverted cone, with air vent at its apex, may be fitted. The size of the cone should be sufficient to raise the bed of hops around it to the desired thickness by blanking off a considerable proportion of the bottom of the back. After the hops have settled, the wort is drawn off, the first runnings being, if necessary, circulated through the hops until a bright wort is obtained. This is then pumped to the cooler or wort receiver and the hops are afterwards sparged with hot liquor to save some of the three-quarter gallon of wort which each pound of spent hops absorbs. Alternatively, but less frequently, the spent hops are pressed. The spargings are pumped up with the other worts to the cooler, but pressings are added to a later copper.

Continental lager brewers dislike a hop back, as the stand on the hops is believed to affect the flavour of the beer. A closed hop strainer is generally used, and the worts are passed through it as rapidly as possible. Hot liquor is then pumped through to wash the extract from the hops and, occasionally, a small quantity of fresh hops is placed in the strainer to add its aroma to the beer. These hops can afterwards be used in the copper. The Montejus form of hop strainer, Fig. 118, is fitted at the top with a sparging ring and also with a propeller for discharging spent hops. Two valves are fitted to the bottom of the vessel, one for wort delivery and the other for the spent hops. After sparging, a small amount of water is added and the spent hops forced out by steam pressure through a pipe to silos or drying plant.

PRINCIPLES OF WORT BOILING

(486) Length and Intensity of Boil.

Boiling time has to be considered from several points of view, including (1) evaporation, (2) extraction of hops, (3) protein coagulation, and (4) sterilisation. Extraction of the resins and flavouring substances from the hops is particularly important in view of its influence on beer character. 1½ hour is adequate to secure the greatest advantage from the preservative substances, and longer boiling times gradually produce harsh flavoured products, particularly with carbonate liquors. (Section 492.) Reference has already been made to the influence of boiling on the hop oils. Sterilisation is certain in this time, and sufficient evaporation ought to be secured. Worts were formerly boiled for much longer periods than is now customary, 1½ to 2 hours representing the normal practice. The change is due to the reduced need for evaporation and the greater delicacy of flavour demanded, with the more efficient plant now available. The boil must in all cases be vigorous and cause a rolling and fountain-like movement of the wort, which greatly increases protein coagulation as well as evaporation.

The rate of evaporation is usually a good guide to efficiency, 8–12% per hour being satisfactory, but the temperature of the wort should also be taken with a registering thermometer, since it is possible in some coppers to get violent movement and good evaporation at about the temperature of boiling water. The temperature of boiling wort increases with its concentration and at greater depths in the copper. That of a wort of 1055 sp. gr. should be about 215° F. at a foot or two beneath the surface and may increase by about 8° at a depth of 9–10 feet, but this, difference is greatly reduced by good circulation.

(487) The Break.

The most striking, visible change produced in wort by boiling either alone or with hops, is the formation of a flocculent coagulum, consisting mainly of one of the chief protein constituents of the malt, albumin. This constitutes what is known as the hot break, to distinguish it from the break that occurs when filtered hot wort is cooled. Its physical condition affords valuable information on the properties of the wort and is a criterion by which the success of the boil can be judged. It should consist of compact, flocculent masses, which settle rapidly in the wort, so that the latter appears clear and black, if left in the copper for a short time after drawing the fire or shutting off steam. It is always accompanied by a certain amount of finely divided precipitate. This should be small in quantity and be carried down with the flocculum. An excessive amount of fine, hot break which, in bad cases, makes it impossible to obtain a bright wort by filtration when nearly boiling, is a sign of under-modified malt or of insufficient ebullition, and is particularly liable to occur when a strongly carbonate liquor is used for sparging. Under these conditions, the weak worts would give a very unsatisfactory break or none at all if boiled alone.

It was pointed out in Vol. I, Section 116, that coagulation of proteins takes place in two stages and that the particles, previously existing in a state of high dispersion, are denatured or dehydrated before they become insoluble and aggregate in smaller or larger flocks. The formation of these flocks and production of a good break is facilitated by active ebullition and formation of bubbles. Protein particles are adsorbed or accumulate on the bubbles and aggregate into larger masses which circulate in the wort as the bubbles burst. Particles which still remain so highly dispersed as to be invisible in the hot wort are mutually attracted and precipitate when the wort cools. The greater quantity of sludge deposited when some types of internal heaters are employed is partly due to increased movement of the wort and bubble formation. The albumin of malt is readily coagulable and the globulin is coagulated to some extent by boiling, but its precipitation and that of higher protein degradation products is largely caused by reaction with tannins (Section 496).

(488) The p_H Value of Copper Worts.

The coagulation of proteins is influenced by the p_H value of the liquid in which the particles are dispersed and is greatest at their isoelectric points. The very varied nature of the protein constituents of wort makes it unlikely that they should have a common isoelectric point, and it has been found that there is little variation in the quantity of nitrogen precipitated from boiling wort within the normal range of p_H values. Hagues[3] found that the amount of protein coagulated during the boiling of infusion mash worts was independent of the hydrogen ion concentration of worts on the alkaline side of p_H 4·0–5·0. Similar results were obtained by Windisch, Kolbach, and Vogl[4] at p_H values between 5·0 and 6·0. The physical condition of the copper break is, however, markedly influenced by the reaction of the wort, and is frequently defective with liquors containing large quantities of carbonates. A p_H value of 5·0–5·2 is generally recognised as most satisfactory. It is also that most suitable for hop extraction (Section 492).

It was pointed out in Section 447, that some of the reactions which influence the p_H value of wort were not brought to completion in the mash tun. This applies, in particular, to those between the phosphates and carbonates in presence of CO_2, and to the precipitation of $Ca_3(PO_4)_2$. Expulsion during boiling of any free CO_2 present in the wort leads to completion of the reaction between bicarbonate ions and hydrogen ions, and should thereby cause a rise in p_H value. The normal reaction, represented by

$$HCO_3 + H \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

and involving the neutralisation of hydrogen ions, is hindered by excess of CO_2. The solubility of tricalcic phosphate is, however, greatly reduced at boiling temperature, so that its precipitation becomes more complete and is no longer appreciably affected by the presence of magnesium and other salts. The acidifying effect of calcium ions is, therefore, more fully exercised and more than counterbalances the basic effect of the carbonates. As a result the p_H value of wort falls by 0·1 or 0·2 during boiling and is stabilised, but too great a fall is prevented by the presence of carbonates. Moufang[5] found that the fall in titratable acidity was appreciably, though only slightly, greater, when the worts were boiled under pressure, and that this was accompanied by a small increase in the phosphate removed, when compared with boiling in an open copper. Part of the p_H fall during boiling is, however, due to extraction of acidic substances from the hops.

Hopkins and Amphlett[6] contend that the precipitation of $Ca_3(PO_4)_2$ occurs almost entirely during boiling, the increase of acidity during mashing being mainly due to precipitation of a calcium salt of phytin. Various factors influence the solubility of $Ca_3(PO_4)_2$. It is less when the p_H value is above 5·3, as in most lager worts, than in the more acid infusion worts, but less phosphate will remain in the latter with increasing concentration of calcium and gypsum. The removal of phosphate during boiling, with liquor rich in calcium, varies with the gravity of the wort, that is with its phosphate content. The loss may be considerable with high gravity worts, while $Ca_3(PO_4)_2$ saturation may not be reached in low gravity worts. There would therefore be little or no fall in p_H from this cause during boiling to counterbalance the high akalinity of spargings. (Section 451.) Since the solubility of $Ca_3(PO_4)_2$ is increased in the presence of magnesium salts at mashing temperature, but not when the liquid is boiled, the hindrance of the comparatively large quantity of magnesium in the malt to acidification of the wort by calcium, which was operative at mashing, is removed during boiling and may account for part of the fall in p_H value. When, in addition, magnesium sulphate is added in any quantity to the mashing liquor, this effect is magnified and the p_H fall during boiling is increased.

(489) Measurement of Coagulum.

A method for measuring the quantity of precipitated material or of coagulable matter left in the wort is useful, on account of the importance of good break and clarification. A photometer can be used to determine the effectiveness of the break by measuring the turbidity of a sample of the wort that has been filtered after cooling. (Bishop and Whitley.[7]) Another method, which has many applications, was described by Kolbach,[8] who determined the quantity of coagulable nitrogen still remaining in boiled wort by reboiling for 5 hours. 200 ml. of the filtered wort is placed in a 500 ml. Kjeldahl flask, connected with a reflux condenser and immersed in a bath of saturated salt solution (40 grams common salt in 100 ml. water). The salt solution is rapidly heated to boiling and boiled for 5 hours at about 226° F. with occasional additions of water to make up for evaporation. The hot wort is filtered and the precipitate on the filter washed once with hot distilled water. The flask is washed out four times on to the filter, which is again washed twice. The filter is returned to the Kjeldahl flask and the nitrogen content determined. A correction is made for the nitrogen content of the filter paper and for nitrogen absorbed by it from wort. This should be found by trial, but may amount to about 0·05 mgm.

A small quantity of coagulable nitrogen always remains in wort after normal boiling periods, and its determination in the manner described provides a means of comparing the efficiency of different plant and processes. Higher results—that is, greater quantities of coagulable nitrogen remaining in the wort—mean less efficient coagulation previous to sampling. Analyses of this kind show a continuous reduction of coagulable nitrogen over several hours' boiling, and support the contention that there is no redispersal of coagulated proteins by excessive boiling. The figures in Table 211, by Kolbach and Wilharm, represent the removal of coagulable nitrogen during a brewery boil of a wort of about 1048 sp. gr. The hops were added after 30 minutes' boiling. Since hops contain about 0·6% of soluble nitrogen, most of which is uncoagulable, the permanently soluble nitrogen of wort may be almost the same before and after boiling with hops at the rate of 2 lb. per barrel.

TABLE 211.—PROTEIN COAGULATION DURING COPPER BOILING (MGM. N PER 100 ML. WORT AT 11·93% PLATO) (KOLBACH AND WILHARM)

Boiling time, minutes	Coagulable nitrogen remaining in wort	Total nitrogen of wort
0	6·0	—
25	4·26	103·0
45	3·64	102·1
60	2·96	100·9
90	2·76	100·6
112	2·40	100·3

Removal of the maximum amount of precipitable nitrogen reduces the tendency to true protein haze (Section 709), but may lead to some loss of fulness. For this reason it may be desirable to make a compromise, when fulness of flavour is the first requirement and moderate stability will suffice. As much protein as safety permits should be left in beer. The choice is of some importance in deciding whether to add all the hops at the commencement of boil or part at a later time, since the nitrogen coagulated is reduced in the latter case. The nutritional requirements of yeast are satisfied by simpler nitrogenous substances which are not coagulated on boiling, so that a thorough precipitation has no detrimental effect in this direction, and is beneficial to the yeast in other ways.

(490) Copper Finings.

An unsatisfactory copper break can frequently be countered by use of so-called copper finings, the clarity of the wort and eventual colloidal stability of the beer being improved. For this purpose the seaweed known as Carragheen or Irish Moss is largely used. This is dark purple or black when collected, but is bleached when dried by exposure to the air and sunlight. It appears to consist essentially of two components, one of which is very soluble in cold water and yields a thick viscous solution. The other is soluble in hot water and tends to gelatinise on cooling. In addition, part of the moss is insoluble and remains as a cellulosic skeleton. The cold water solution consists of carbohydrates united with calcium and sulphate, with traces of magnesium, iron, etc. Gelatinisation is largely due to the starch-like carbohydrate, alginic acid, formerly known as lichenin. As supplied for use, it contains about 17% of moisture and, like many denizens of the sea, is liable to contain a slight trace of arsenic, which makes careful selection necessary. Weighed quantities, from which extraneous sand has been removed by teasing and shaking, can be added direct to the copper. Preliminary washing removes a considerable proportion of the soluble constituents. It is also available in purified, powdered preparations. Care must be taken to add the correct quantity for maximum fining action. Excess is liable to defeat this object, as it appears to act protectively to the colloidal particles which it is intended to remove.

Ranken[9] found that flocculation in boiling wort is practically uninfluenced by the concentration of nitrogenous and other wort constituents, but that it is sensitive to the reaction of the wort and to the concentration of the moss per barrel of wort. He made experiments with 0·2% solutions of peptone to find the optimum conditions for fining action. The optimum p_H was found by comparing the clarity of filtrates after boiling with weighed quantities of Irish moss at different reactions and found to be at p_H 5·3, 5·0, 4·8 and 4·5 for rates equivalent to 1½, 3, 4½ and 7 lb. per 100 barrels. 1½ lb. per 100 barrels did not produce maximum clarity with the more acid solutions, but excess is to be avoided, as the increase of acidity during fermentation is liable to produce a further gelatinous precipitate. Usual rates in practice are between 1½ and 3 lb. per 100 barrels of wort. The purified active constituent of Irish Moss is now commercially available, under the name of Manucol.

(491) Effects of Aeration.

The absorption of oxygen during boiling is hindered by the steam, but there can be little doubt that it does occur to some extent and results in the oxidation of wort constituents, with increase of colour. The transformation of tannins to dark coloured phlobaphenes is accelerated in presence of air, and there is also an increase of colour when humulon is transformed into resins by boiling under similar conditions. (Windisch *et al.*[10]) The production of paler worts in covered coppers has been explained by the exclusion of air. These reactions with oxygen do not imply that there is any considerable solution of air during boiling, and most of that absorbed in mash tun wort would be driven off. Their effects on the colour of wort are much less than those due to the caramelisation of sugars or their combination with amino-acids to produce melanoidins, which are accompanied by changes of flavour that are desirable in some dark beers and are deliberately encouraged. They are also of less significance than the colour change resulting from the use of carbonate liquors and associated with a high p_H value of the wort. (See also Section 504.)

EXTRACTION OF HOPS

(492) Resinification of Humulon.

The nature of the changes undergone by the resin constituents of hops during wort boiling is still only incompletely known and has been somewhat obscured by use of the term α-resin to designate a fraction that gives a precipitate with lead acetate in methyl alcohol solution. This fraction consists essentially of humulon and constitutes about 40% of the "soft resins" of good quality hops. It is insoluble in liquids having a p_H value corresponding with that of beer and, hence, it is of interest to discover how its flavouring and preservative properties are made use of. This must be due to chemical changes in the copper, which cause some part of it, at least to, become soluble without complete loss of antiseptic value and flavour. The hard resin produced from humulon is apparently an oxidation product, but its transformation to soft resin can be caused by boiling in aqueous liquids in absence of air. The " boiling product " (Section 273) probably has the same molecular weight as humulon, which it resembles in being bitter and antiseptic. It is more strongly acid and more soluble in wort than humulon and, since the latter is insoluble in beer, its production would appear to be essential in copper boiling. A different soft resin is produced when humulon is resinified in presence of air, as may happen to some extent in the copper. This is known as " oxidation product." Both these α-resins are included in the β-resin or β-fraction in gravimetric analyses, since they do not give precipitates with lead acetate in methyl alcohol.

Two methods have been used to investigate the behaviour of humulon in boiling wort. In one (Kolbach and Kleber[11]), the quantities of unchanged humulon, originally added to wort in alcoholic solution, and of its resinous products recoverable after boiling by extraction with chloroform were determined by gravimetric analysis. In the other (Walker[12]), estimations of the changes in antiseptic potency of the wort have been made by the log phase method. (Section 271.) These have both been helpful in elucidating the transformations of humulon, but little has yet been ascertained about the corresponding changes of the other bitter acid, lupulon, which are probably of a somewhat similar nature.

An estimation of the rate of resinification of humulon, under conditions that prevent complication by oxidation and removal by adsorption on coagulated proteins, showed that 79·8% of the original humulon could be recovered in an unchanged form after boiling in wort for 15 minutes, 59·4% after 60 minutes and 37·6% after 2 hours. The remainder would be changed to α-soft resin, "boiling product." The conditions are different when humulon is boiled in wort from which the coagulable protein had not previously been removed by boiling, but still in an atmosphere of nitrogen. Under these circumstances a considerable proportion of resin and humulon is adsorbed on and removed with the coagulum, as shown in Table 212. It was found, by adjusting the p_H value of the wort with HCl or NaOH, that resinification was increased at higher p_H values, but the influence of hydrogen ion concentration was small in comparison with that of boiling time. A proportionally smaller quantity of humulon was, however, removed with the coagulum in the more alkaline media. The wort contained 6·51 mgm. coagulable nitrogen per 100 ml. The quantity of humulon added, in the form of alcoholic solution,

TABLE 212.—INFLUENCE OF THE p_H VALUE OF WORT ON THE REMOVAL AND RESINIFICATION OF HUMULON DURING BOILING FOR 1 HOUR IN WORT (KOLBACH AND KLEBER)

(PERCENTAGES OF ADDED HUMULON)

p_H of wort	α Resin found in		Humulon found in	
	Wort	Coagulum	Wort	Coagulum
5·26	38·3	9 7	26·8	18·2
5·57	40·9	12·9	25 0	14·7
5·85	42·7	15·1	23·5	12·3

corresponded with a hop rate of about 1 lb. per barrel, the hops containing 6% of humulon.

It appears from these experiments that the greater bitterness of beers brewed with carbonate liquors depends more on the greater solubility of the resins in alkaline media than on increased resinification of humulon. The greater quantity of coagulable nitrogen in infusion mash worts would remove more humulon and resins and account, in part, for the higher permissible hop rate.

Kolbach and Kleber's analyses showed that no unchanged humulon existed in beer. Any that survives copper boiling and cooling is precipitated in the fermenting vessel or adsorbed on the CO_2 bubbles and yeast. It is, consequently, only of service in beer in so far as it is converted to α-soft resin. The proportion so converted depends mainly on the time of boiling. The analyses recorded in Table 213 confirm earlier findings that the amount of soft resins remaining in the beer increases with boiling time and approaches a maximum in about 2 hours. They show also that the quantity of resins removed on the yeast, etc., correspondingly decreases. The extraction of resins from yeast by chloroform is difficult and a good balance between original humulon and recovered resins was never obtained.

TABLE 213.—INFLUENCE OF BOILING TIME ON THE RESIN CONTENT OF BEER (WORT, 12·33% PLATO, p_H 5·72) (KOLBACH AND KLEBER)

Boiling minutes	Beer p_H	Resins in beer % of added humulon		Resins in yeast, etc. % of added humulon	
		α-resin	Humulon	α-resin	Humulon
5	4·51	21·3	0	7·8	40·6
15	4·51	24·7	0	7·7	39·2
30	4·51	38·1	0	6·9	28·7
60	4·48	49·9	0	9·8	15·3
120	4·47	52·5	0	11·2	10·3

(493) Extraction of Resins from Hops.

The extraction of resins from the hops is a comparatively slow process, complicated by their colloidal character, tendency to form adsorption complexes with proteins and by the readiness with which some of their constituents undergo decomposition. It probably takes place in two stages, in the first of which the resins are melted by the heat of the wort and form droplets which are afterwards dispersed in a fine state of division and, in part, adsorbed on protein particles. If the latter are coagulated by boiling, the adsorbed resins are removed with them. Walker found that the antiseptic potency of the wort at first rose rapidly, as the hop resins were dispersed in it. The maximum preservative value was attained after boiling for about 45 minutes. After this it falls, but tends to become constant in about 90 minutes, at a figure which is very approximately 40% of the preservative value of the humulon content of the hops. This is due to the balance between the gradual extraction of preservative substances and their progressive decomposition and removal on coagulated proteins.

Kolbach and Kleber's experiments were extended to actual brewery boils. The results are probably fairly typical of what occurs in the copper and suggest various deductions of practical significance. The experiments were carried out in two breweries.

Brewery A.—Hop rate of 200 grams per hl. (about 0·78 lb. per barrel). The mixed hops contained 6·5% humulon, 8·1% β-fraction. All the hops were added when the last worts were collected and were boiled for 1½ hour. They would add 19·5 grams humulon and 24·3 grams β-fraction per hectolitre.

TABLE 214.—RESINS IN WORT AND BEER. BREWERY A
(PER CENT. OF ADDED RESINS OR HUMULON)
(KOLBACH AND KLEBER)

	In Wort or Beer		In Coagulum	
	Resins	Humulon	Resins	Humulon
Wort, boiled 15 minutes ..	16·6	24·4	29·2	5·7
,, ,, 60 ,, ..	28·1	21·6	36·1	10·0
,, ,, 90 ,, ..	35·2	9·5	41·5	7·4
Collected wort	33·5	0	12·5	9·5
Fermented 3 days	19·8	0	—	—
,, finished ..	19·0	0	—	—

Brewery B.—Hop rate 320 grams per hl. (1·15 lb. per barrel). The hops contained 5·7% humulon and 7·4% of β-fraction, adding 18·24 grams of humulon and 23·68 grams β-fraction per hl. Three-sevenths of the hops were added as soon as the bottom of the copper was covered, four hours before boiling commenced. Two-sevenths were added at the commencement of boil and two-sevenths one hour before turning out. The total boil was 95 minutes.

The results show the slow extraction of resins, removal of a considerable proportion with the coagulum and progressive resinification of humulon. A small quantity of resins was thrown out during cooling. The presence of unchanged humulon with the resins adsorbed on the sides of the fermenting vessels and yeast was established, but no quantitative estimation could be obtained. It will also be observed that no humulon was found in the beer. The worts at Brewery B contained more resins and humulon after 15 minutes' boil than those at A, on account of the long preliminary soaking of the hops in hot wort. The third portion of hops added only a trifling quantity to the resins extracted, this being particularly marked in respect of humulon. Despite the greater quantity of humulon and resins added at Brewery B, the total recovered in the boiled worts was almost the same at both breweries, 119·3 mgm. per 100 ml. at A and 114·9 mgm. at B. In contrast with this, the resins in the coagulum at B were more than at A. Addition of a portion of the hops late in the boiling period is evidently not economical, in so far as quantity of resins dissolved by the wort is concerned. Its advisability must be judged from the point of view of flavour.

Table 215.—Resins in Wort and Beer. Brewery B
(per cent. of added resins or humulon)
(Kolbach and Kleber)

	In Wort or Beer		In Coagulum	
	Resins	Humulon	Resins	Humulon
Unboiled wort + hops 1 ..	12·5	12·2	5·8	9·1
Boiled 15 mins., Hops 1 and 2 ..	19·9	25·8	12·8	10·2
„ 60 „ „ 1, 2 and 3	21·9	9·8	25·8	15·0
Wort boiled 95 minutes ..	23·2	2·7	31·6	11·6
Collected wort	22·4	0	—	—
Fermented 2 days	17·1	0	—	—
„ finished	16·2	0	—	—

Other determinations on similar lines showed that the quantity of resins dissolved or precipitated during boiling varied with the protein content of the wort. A wort with high nitrogen content holds more resin by adsorption but, on the other hand, more coagulable protein causes a greater loss during boiling. The resin content of wort is not proportional to the hop rate., The percentage destroyed and precipitated is greater with increasing hop rate. Less resin is retained in more acid worts, but there is subsequently less loss during fermentation. The adsorption of bitter substances on wort proteins or protein digestion products which are not coagulated in the copper may have a far-reaching effect on beer flavour. If these are not adequately removed during cooling, but remain in colloidal dispersion in the beer, they are liable to be precipitated during storage and cause the harsh bitter flavour of beers which have become turbid in this way. Removal of substances that behave in this manner is an important object of boiling and cooling and can only be carried out adequately when the copper break is satisfactory. On the other hand it is necessary to ensure an adequate bitterness in the beer by the presence of hop extractives in stable colloidal dispersion. That time is necessary to convert the humulon into the more soluble transformation products and bring the resins into such a state of dispersion that they will not be removed by precipitation or adsorption during fermentation, is shown by the figures in Table 216, representing the total resins extracted from wort by chloroform before and after fermentation. (Kolbach and Vogl.[13]) An optimum boiling time of 1½ or 2 hours is suggested.

TABLE 216.—BOILING TIME AND RESINS IN WORT AND BEER (KOLBACH AND VOGL)

Time of Boil minutes	Resins in Wort mgm. per litre	Resins in Beer mgm. per litre	Loss during Fermentation mgm. per litre
10	122	49	73
60	126	92	34
120	127	97	30

(494) Influence of Boiling on the Preservative Power of Humulon and β-Soft Resin.

A striking point in Walker's experiments, in which alcoholic solutions of humulon were added to wort at 149° F., was that up to 40% of its original antiseptic potency was immediately rendered inoperative and that more was lost during the six minutes required to bring the wort to the boiling point. The reason for this cannot be definitely stated, but it was ascribed partly to adsorption on the proteins. Part may be due to removal of undissolved humulon and part arises from removal with coagulated proteins during the six minutes of rising temperature. Part may also be due to transformation to less powerfully antiseptic resins. Some 50% of the remaining preservative value is destroyed by transformation of the humulon and adsorption on coagulated proteins during 2 hours' boil, so that a boiled and filtered wort only retains about 35 or 40% of the preservative potency of the humulon originally added. This closely corresponds with the quantity of humulon and resins found in boiled worts by

Kolbach and Kleber. Walker also found that the rate of loss was much greater during the earlier part of the boiling period and gradually decreases until a point was reached at which the preservative value of the wort per unit of volume commenced to rise, on account of evaporation and volume shrinkage. Increase in the rate of boiling resulted in increased rate of destruction, but the initial p_H value of the wort had little influence on its preservative value at the completion of boiling.

Walker's most recent investigations, of which he has privately communicated a resumé, indicate that the preservative value of β-soft resin is destroyed to the extent of 95% in the copper. The residual 5% is lost during fermentation. Hence the β-soft resin of copper hops is valueless as a preservative agent in the beer. The preservative value of a beer, excluding any due to dry hops, is usually about 10% of that of the humulon content of the hops used in the copper, provided that the hop rate is low or medium. The losses of preservative value appear to be greater with a high hop rate. No direct proportionality was detected between the preservative values of the beers and the humulon contents of the hops used.

(495) Influence of Resins on Foam Stability.

Boiling with hops increases the foaming capacity of worts. This is on account of the extraction of resins, which reduce the surface tension of the wort and themselves tend to collect at its surfaces with air and the containing vessel or on the surfaces of bubbles. (Vol. I, Sections 83 and 86.) The resins form a thin skin on the bubbles, thereby greatly increasing their stability. Peard and St. Johnston[14] found that the surface tension of all-malt hopped wort, taken from the refrigerator, varied with its specific gravity from 46·3 d/cm. at 15·5° C. for wort of 1142 sp. gr. to about 52 d/cm. at 1050 sp. gr. and 56 d/cm. at 1040 sp. gr., as determined by Sugden's maximum bubble-pressure method, the surface tension of water being 75 d/cm. The surface tension was also found to vary with the p_H value of the wort, showing a maximum at p_H 5·4, with a gradual decrease on both sides of this, but only up to p_H 6 on the alkaline side, after which it again rises. The stability of beer foam depends on the presence of protein derivatives and probably other substances, but it is probable that hop resins also contribute to it.

(496) Tannin Reactions.

The tannins, as a group, have characteristic properties which suggest that any extracted from malt husks or hops should play some part in the coagulation of proteins during wort boiling.

They are readily soluble in water and are converted to dark coloured and only slightly soluble condensation products, known as phlobaphenes, when their solutions are heated, particularly in presence of acid or air. This appears to occur in the copper and contributes to the darkening of colour when worts are boiled. Tannins and phlobaphenes are negatively charged and combine with positively charged proteins. The coagulation of albumin, which constitutes the major part of the copper break, is probably only influenced to a minor extent by reactions with tannins, but the production of adsorption complexes with protein derivatives of high and medium complexity has far-reaching effects. Two types of protein-tannin combinations have been described, the term protein here including its degradation products, giving what are frequently called peptone-tannins. These are the protein-tannins and the protein-phlobaphenes. Representatives of both types are produced in the copper. The latter are insoluble, so that they are precipitated during boiling, and the greater precipitation of nitrogen by intensive boiling is probably associated partly with greater conversion of tannin to phlobaphene by oxidation. The tannin compounds are soluble in hot wort, but are precipitated on cooling and redissolved when the wort is again heated. That is to say, they are reversible. They are, consequently, deposited with the fine sludge during cooling, but this precipitation is slow and incomplete. It continues during fermentation, storage and in the finished beer, giving rise to haze. The relative proportion of tannin and protein in these complexes varies, because the original proportions of tannin and protein vary. They also contain iron and adsorbed silica, which are precipitated with them.

It was suggested by Schjerning and also by Hartong[15] that the protein part of the protein-tannins was derived from the small quantity of globulin that remains in wort in the form of hydrated adsorption complexes with mineral matter, particularly P_2O_5 and silica. The tannin may be derived either from the hops or malt. It is probable that only a comparatively small quantity is extracted from the malt husks in an infusion mash, and that most of the tannin comes from the hops. Considerably more is derived from the malt, which contains about 0·3% tannin, in decoction systems on account of the boiling to which the mash is subjected and the lower hop rate of lager beers leads to smaller quantities of tannin from this source than in ales. According to Hartong,[16] who used a method for estimating the tannin in wort, based on titration with 0·01N $KMnO_4$ in presence of indigo, before and after treatment of the wort with a tannin adsorbent, the unhopped wort of a pale lager beer of about 1048 sp. gr.

contained 0·0111% of tannin, derived from the malt, while the hops added 0·0080%. After boiling, the total quantity was reduced to 0·0166%. During primary fermentation, the quantity of tannin fell to 0·0157% and, after 2½ months' storage of the beer, to 0·0152%. These results indicate that wort derives more tannin from the malt in a decoction mash than from the hops, and that the proportion removed during boiling, fermentation and storage is comparatively small. It might be mentioned that some authors (Heintz[17]) contest the importance of tannin in connection with cold sludge and beer haze, but the opposing views probably arise from differences in the definition of tannins.

(497) Summary.

The extract and composition of the wort and its fermentability are determined in the mash tun, but copper boiling plays a greater part in deciding the character and stability of the beer. These largely depend on the extraction of flavouring and preservative substances from the hops, and removal of complex proteins by coagulation, for which an energetic boil is essential. The time of boiling does not so much depend on sterilisation and concentration requirements, as on that necessary to bring colloidal constituents of the hops into dispersion and ensure the stability of the solution by adequate chemical and physical changes in the resins and proteins. Every brewer knows how essential it is to bring about a happy " marriage " between the bitter substances of the hops and sweet derivatives of the malt. This depends, firstly, on finding suitable partners. The kind of hops and their quantity are carefully selected in accordance with the type of wort and kind of beer to be produced. The marriage is then made in the copper and seems to be forced upon the humulon of the hops. The latter is not easily brought into intimate union with the wort. About 1½ hour boiling is required to effect this and, in the process, about half its preservative activity is dissipated by association with other colloids, and only about 40% remains in union with the intended partner. A large part of the humulon is resinified and, thereby, becomes more amenable to permanent bondage with the wort. Despite this coercion, part of the humulon still retains its original freedom and escapes during fermentation by precipitation on the vessel or the yeast, in association with protein matter. Humulon is not soluble in beer, and is only useful in so far as it has been transformed to more soluble resinous products, which still retain preservative activity. The bitter flavour of the humulon is increased as it resinifies, and is modified by union with saline constituents of the liquor, becoming increasingly unpleasant by consorting with carbonates. Its antiseptic partner, lupulon, appears to be completely destroyed by boiling.

The other partner to the marriage has very objectionable possessions, and it is only by energetic boiling that these highly complex proteins are thoroughly coagulated and eliminated. There are, however, simpler protein derivatives which contribute to the fulness of beer, but some of these unite with tannin from the hops to produce the so-called peptone-tannins, of which more will be heard in the next Chapter. They are, in large measure, removed from the wort during cooling and fermentation, but some refuse to leave the beer and produce a definite instability, marked by their unwelcome appearance as haze or turbidity. The question of the utility of the tannins naturally arises. Are they of greater service in removing the protein derivatives of medium complexity, or more detrimental through their uncertain behaviour when combined with the latter ? This is difficult to answer, but there is no doubt that the tannins do cause a lot of trouble to brewers, and probably contribute a share to the different behaviour of mild and bitter beers and increase the tendency of the latter to become cloudy.

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CHAPTER XXIX

WORT COOLING

PRINCIPLES OF COOLING

(498) Objects of Cooling.

The primary object of cooling, reduction of the temperature of boiled wort to that necessary for fermentation, can be attained by its exposure in shallow layers to the atmosphere. This, sometimes with the help of submerged coils for circulation of cold liquor, was the method invariably adopted until Pasteur and Hansen pointed out the dangers of infection by bacteria and wild yeasts. The open cooler was the most obvious point of infection and safety appeared to involve its abandonment or modification of the usual process of cooling. Improvements were made in refrigerators, and many brewers replaced their coolers by wort receivers, commanding refrigerators on which the wort was rapidly cooled, with much less exposure to aerial infection. Others restricted cooling on the shallow open backs to about 140° F., which few micro-organisms in a vegetative state can withstand, and completed cooling on refrigerators. Attempts were also made to devise completely enclosed refrigerators. In some cases, the change resulted in defective fermentations or cloudy beers, which were attributed to insufficient aeration or failure to remove wort constituents that ultimately caused haze in beer. The latter difficulty, whether due to this or other causes, became increasingly obvious as the demand for brilliant beer expanded. The requirements of a satisfactory cooling process consequently include :

(1) Reduction of temperature, with freedom from the danger of infection.
(2) Removal or facilitation of the subsequent elimination of haze-producing wort constituents.
(3) Sufficient aeration to satisfy the requirements of yeast in the early stages of fermentation.

Combination of all these objectives in an efficient and economical cooling plant did not prove a simple matter, because some of the factors concerned in their attainment were unknown and their relation with defects encountered have only gradually been elucidated. Experimental results have sometimes been wrongly interpreted and have led to tentative designs which, though they may have attained their immediate objects, have had to be abandoned or modified as new information was gained or other defects became apparent. It can hardly be claimed that cooling plant has yet reached its maximum efficiency from all points of view or that all the factors which must be met are known and fully understood.

(499) Infection during Cooling.

The death-point of micro-organisms varies with the species and may be raised 10° or 20° F. with yeasts when spores exist. Thus young cells of *S. validus* are killed in 5 minutes in water at 133° F., but older cells may survive 140° and spores 150° or even higher temperatures. Dried spores of some other micro-organisms can withstand boiling for some hours. These figures suggest that security is not obtained by limiting cooling on open vessels to a temperature of 140°. This is the generally accepted minimum, but it is difficult of realisation with the last few barrels run down. Most vegetative cells may be killed at that temperature, but some may survive, as many spores will. A large proportion of the surviving micro-organisms, most of them weakened by previous desiccation, are destroyed or suppressed during fermentation, but some of the latter may regain their vitality during storage, when the primary yeast activity is spent. In cases of serious infection, the effects on the beer, some days or weeks after racking, may be very detrimental. Despite the fact that brewers in more favoured localities can use open coolers without fear, the risks are there in many places and force the choice between their elimination by means described in later sections, and abandonment of coolers, which involves finding alternative methods for securing advantages which exposure in shallow, open vessels offer.

(500) Flocculation of Wort.

Among the advantages of a shallow cooling vessel is the opportunity it provides for deposition of flocculated protein matter, the " hot break," carried from the copper through the hop strainer. This service is not required of the cooler when the copper break is good and an efficient filtering hop bed is provided, as it is in a good hop back, particularly if provision exists for circulating the wort through the hops until it runs brilliant. The brilliant wort, however, becomes turbid when cooled. In some cases clouding commences at 150° F. or at higher temperatures, but other worts remain bright down to about 130°. The turbidity generally attains a maximum between 100° and 80° F., but precipitation continues more slowly after this. It is due to finely divided colloidal particles, consisting mainly of protein matter in a more or less degraded state, derived from the malt, combined with tannin from the hops and malt. (Section 496.) The so-called protein-tannins are soluble in hot wort, but become insoluble at lower temperatures and carry down with them a small quantity of silica and other substances. They differ from the proteins coagulated in the copper, in that they redissolve when the wort in which they are precipitated is warmed. The particles are extremely minute, ranging from 5 μ (0·005 mm.) in diameter down to the limits of microscopic vision, and cannot be separated from cold wort by filtration or centrifugation, unless they are previously caused to aggregate or flocculate. Appearance of such aggregations in cooled wort is called the " cold break." It is not usually so flocculent as the " hot break," for which reason the copper break and that produced at the lower temperatures are frequently distinguished as the " coarse " and " fine " break, respectively, but it would be preferable to abandon these terms and restrict the qualifications, coarse and fine, to use with both " hot break " or " cold break," because the aggregations in either case may vary from a flocculum to a powdery form. " Cold break " then occurs when the invisible particles producing turbidity aggregate to such an extent as to become visible as discrete masses of " coarse cold break " or " fine cold break."

The flocculation or aggregation of colloidal particles may be hastened or improved by various physical conditions or mechanical operations, but precipitation and flocculation of the colloids of wort is not completed in the short time occupied by cooling, and continues during fermentation and even in the finished beer, causing haze. It is desirable that as much as possible of the haze-forming protein-tannins should be removed before the beer is racked or bottled and, if possible, during cooling and with the early yeast heads, rather than by slow precipitation during fermentation or storage. The best sign that this will be effected is good and rapid flocculation or break in the cooled wort. The problem has been to find the factors that facilitate break and apply them in cooling plant, without detriment to other requirements. Complete clarification of worts is not desirable, but difficulties during fermentation and with the finished beer are liable to arise when they are opalescent, with fine particles in suspension, on account of defective break.

(501) Results of Defective Break.

The first consequences of defective cold break are the passage of a large proportion of the sludge that might be retained in the cooling plant to the fermenting vessels, together with excessive or delayed precipitation therein of finely-divided protein-tannins with adsorbed hop resins. Among the defects that result are,

(1) A harsh bitter flavour in the beer, which is sometimes erroneously attributed to the hops, caused by solution of finely divided protein-tannin particles during suspension in the fermenting vessel, particularly as the temperature and acidity of the wort rises. The adsorbed resins are liberated, but increasing acidity reduces their solubility, so that they separate, in large measure, on the yeast or sides of the fermenting vessel, though they may also contribute to the harsh flavour of the beer.

(2) A more or less persistent haze in the beer, with a tendency to incomplete response to finings or filtration difficulties. This is due to finely divided material that remains in suspension after fermentation.

(3) Signs of yeast weakness may be detected during fermentation, with unsatisfactory attenuation. This is generally attributed to adsorption of colloidal material on the surface of the yeast cells, but the actual existence of adsorbed substances has not been proved.

(4) The beer may be sluggish in after-conditioning and more than usually prone to development of wild yeast and frets during storage, on account of the check to primary yeast activity.

(5) In addition to defects due to the presence of finely divided suspended matter, there is an increased tendency to early development of haze in the finished beer, particularly noticeable in bottled, filtered beers. This results from failure to precipitate and remove colloidal protein-tannin particles which, though sufficiently dispersed to have no influence on the brilliance of wort or beer at first, tend to aggregate under the influence of cold, agitation or change in p_H value. This is the ultimate cause of the non-biological hazes in beer, dealt with in Section 709.

A test should be regularly carried out in the routine of brewing to discover whether the break has been satisfactory. The appearance of a sample of the wort, taken from the refrigerator pan and set up in a cylinder, after standing for a few hours, gives a good indication of the nature of the break, but it should be filtered after standing overnight. A bright filtrate cannot easily be obtained with a badly flocculated wort. The appearance of the wort, if filtered immediately, is not always a reliable indication of the flocculation that would occur on standing.

(502) Factors Influencing Flocculation.

It is necessary to go back to the malt, to examine the liquor composition or treatment and the conditions of mashing in a search for the factors that influence the cold break, and it is found that some of them correspond with those that affected the copper break. Wort from badly modified malt or from brews with wrongly treated liquor, imperfectly flocculated in the copper, carries an excessive amount of finely divided suspended matter, which will pass through the hop back and not deposit completely during cooling, even when an open cooler is used, though it gives a large amount of cooler sludge. The further precipitation of protein-tannin during cooling is similarly affected. It is incomplete and the turbidity refuses to break, either during cooling or in the fermenting vessel. Deposition of calcium oxalate or presence of a large proportion of dextrins and high molecular protein degradation products, capable of acting as protective colloids, may hinder separation of the colloidal particles. The latter are constituents of a constantly changing colloidal system. The attainment of equilibrium by precipitation of unstable constituents is influenced by the composition of the various components of the system and is facilitated by increasing hydrogen ion concentration of the wort, but is known to depend on certain physical factors operating during reduction of temperature, many of which can be favourably influenced by design of the cooling plant or rate of cooling.

The design of wort refrigerating plant has been greatly influenced by the investigations of Horace Brown[1] (1913) and others at later dates. Brown found that most of the precipitation occurred between 120° and 80° F., the "critical cooling range," and that the best flocculation was produced by passing a current of air through wort as it cooled through this range of temperature. Inert gases produced the same effect, showing that it was not due to oxidation. The conclusion was that agitation over the critical range of temperature was the essential factor. Brown was also led to believe that the time occupied in cooling through this range should not be less than 30 seconds. Moritz[2] (1921) urged, in opposition to the view that agitation was essential, that length of travel and smooth running of the wort in thin bands over the refrigerator were the main factors in securing flocculation. Emslander[3] (1922) held, on the other hand, that the type of plant and manner of cooling were of only minor importance in the flocculation of wort. This, he contended, depended on the ability of such colloidal substances as protein-tannins to reduce the surface tension of the liquid in which they existed and concentrate at the interface between the liquid and air. Thus a skin forms on hot wort as it cools to 140° on open coolers. This should be broken occasionally into flocks, but not continuously agitated. The wort should remain at rest below 140° F. in order that these flocks may deposit, carrying with them adsorbed fine precipitates. Efforts have consequently been made to design refrigerators or operate open coolers in such a way as to put these findings into practice, first, following Brown, to cause agitation on refrigerators and, later, to extend the travel, as Moritz suggested, or surface exposure of wort to metal or air, according to Emslander's view, and attain, as nearly as possible, a cooling time of 30 seconds between 120° and 80° F.

(503) Speed of Wort Refrigeration.

Considerable doubt was thrown on the generally accepted opinion that wort must be held for as long as 30 seconds over the critical range of temperature by Clendinnen's[4] observation in 1935 that more rapid cooling, far from having a harmful effect on flocculation, was, with some worts at any rate, an essential for good break. Clendinnen noticed that flocculation occurred in certain brewery worts between 140° and 70° F. and that marked differences were observed in the break with slight increase in the time occupied in cooling through this range in different breweries. For example, there might be a good break in wort cooled through this range in 3 to 5 seconds, but none when the time was extended to 9 seconds or more. That is to say, the wort remained cloudy after standing over-night. Clendinnen referred to this interval, 5 to 9 seconds in this case, in which there was a marked change in break as the " critical break rate." He found, however, very remarkable differences in the critical break rates of different worts. In one brewery there was no break in some worts when the cooling time over 140° to 70° F. was greater than 3 seconds, which meant very rapid cooling. In other worts in the same brewery, the critical break rate was between 12 and 17 seconds, a medium rate of cooling, while in a wort from another brewery, the change from good break to no break occurred between 25 and 40 seconds. It broke well at 25 seconds, but not at all at 40 seconds. This is distinctly slow cooling, and cannot be attained on an ordinary refrigerator.

Clendinnen's main conclusion that the rate at which wort was cooled between 140° and 70° F. had a marked effect on the flocculation in the cold wort and that there was a limited " critical break rate " for most worts, than which faster rates gave good break and slower rates no flocculation, was confirmed in laboratory experiments by Bishop and Whitley[5] and Moritz[6] and by trials in a Paraflow refrigerator by Watkins,[7] whose results were summarised by Seligman[8] as follows :—

(*a*) Worts which flocculate well when slowly cooled, that is within 16 seconds between 120° and 80° F., as in the normal Paraflow, are not prevented from flocculating when rapidly cooled in 3–6 seconds.

(*b*) Many worts which do not flocculate when slowly cooled, do so when cooled rapidly.

(*c*) Some worts do not flocculate even when rapidly cooled. Watkins, however, pointed out that variations existed in the optimum temperature ranges and times, depending on the worts and conditions of temperature, as Clendinnen had found. Bishop and Whitley interpreted Brown's original results as the effects of speed of cooling, due to the air current, rather than to agitation, while Moritz attributed the clarifying effect of horizontal refrigerators to the large surface of cold metal to which the nearly boiling worts were exposed.

Shimwell, Kirkpatrick and Baylis[9] emphasised the difference in cooling time required to produce a good break with different worts. They found that worts from a particular brewery, that at which many of Brown's original results had been obtained, always exhibited the best break when cooled slowly, while some laboratory worts from the same materials behaved in a similar manner, but that others flocculated best when rapidly cooled. No definite times were given, but speeding up resulted in bad flocculation with the slowly breaking worts. The brewery control consisted in comparison of the break in tubes of hot wort taken from the cooler and allowed to cool slowly at room temperature with similar samples taken after rapid cooling on the refrigerator. These authors found that agitation during cooling was of great importance, but influenced flocculation differently in the two types of wort. Flocculation of " slow type " worts was adversely affected or even prevented by agitation during cooling, but this had a beneficial effect on " fast type " worts. Gypsum, at the rate of 25 grains per gallon, greatly improved the flocculation of slow type laboratory worts. Additions of salt to the liquor appeared to have little or no effect. Hop rates of 1, 2 and 3 lb. per barrel progressively increased the amount of turbidity produced on cooling and adversely influenced the break.

The results of these laboratory experiments and brewery trials indicate that time of cooling is an important factor in flocculation, but that considerable differences occur in the optimum rate in different breweries. The relative advantage of slow or rapid cooling and the best conditions must, therefore, be found in each case by experiment. Many brewers consider that the best results are obtained with an open cooler and, when such is the case, the optimum range of time is probably comparatively long, since cooling from about 140° F. to pitching temperature is rather slower than cooling from 190° F. on the same refrigerator.

(504) Hot Aeration.

It has been very generally held, since Pasteur demonstrated the apparent effects produced by hot aeration of wort, that oxidation of some of the wort constituents occurred on the cooler and promoted sedimentation and brilliance. He ascribed this brightening effect to modification of the physical structure of soluble resinous substances which thereby became insoluble. Though originally dissolved in sugar solutions, these would precipitate when the sugar fermented. Others have found that maltose, glucose and fructose solutions, proteins and hop extracts absorb oxygen and get darker, and that twice as much oxygen is absorbed at 180° as at 113° F. Actually, very little is known about the effect of oxygen on hot wort, either in regard to the nature of the substances which may undergo oxidation or its influence on the properties of the wort. Practical trials on coolers, with and without aeration, have indeed appeared to support the contention that aeration increases precipitation. A brewer who obtained 80 kgm. of cooler grounds from 230 hl. when the wort was allowed to cool without aeration found that the quantity was increased to 120 kgm. when a vigorous air current was passed for 2 hours over the surface of the wort during cooling. Sufficient time was allowed after passage of air for the sediment to settle firmly before the wort was run down.

Explanations, other than oxidation, can, however, be advanced for the improvement in sedimentation effected by air currents, and other brewers, with facilities for aeration, find no advantage and have dispensed with it. Oxidation can probably be completed by circulation of the worts through the hop back, if not in the copper. Brown's experiments on wort cooling showed that flocculation did not depend on oxidation, since equally good results were obtained by passing a stream of hydrogen through the wort. Ranken[10] found that the only measurable effects of oxygen absorption by hot wort were increase of colour, due to combination with some of the tannin compounds and, possibly, impairment of the flavour of the beer. Oxidation of tannin contributes to its conversion to the coloured phlobaphenes. Lovibond found that a wort, leaving the hop back with a colour of 12·7, darkened to 16·0 on reaching the closed cooler after injection of air in the rising main, and left it after 30 minutes with a colour of 18·0.

Van Laer's[11] experiments indicate that equal weights of hop resins and fructose combined with much more oxygen than tannin, and still more than maltose or glucose. He concludes that fructose, despite its small quantity, accounts for 50% of the oxygen absorbed by wort, which amounts to about 7 ml. per litre of wort per hour. Oxidation of the sugars produces compounds of acidic character. The oxidations and increase of colour are more intense at high p_H values and, despite the surface exposure during colour, are probably more significant during boiling. The amount of sugar oxidised from mashing to cooling must be too small to have any marked effect on the chemical composition of wort.

(505) Agitation on Coolers.

The alternative explanation of the apparently beneficial effect of aeration on the cooler, referred to in the last Section, is afforded by consideration of the influence of agitation on the behaviour of separated colloidal particles. These tend to form a skin on hot wort as it cools to 140° F. (Section 502), and this is broken into flocks by the surface agitation caused by a current of air. They then sink in the wort and carry down adsorbed particles of very fine sludge. This action is much more pronounced on shallow open coolers than in deep wort receivers, accounting in part for the improved clarification of wort on the former. Propellers are fitted on many coolers to stir up the deposited coarse particles and cause them to adsorb the finer and some brewers stir the wort with rakes for the same purpose, a sufficient time being allowed for settlement in either case. These devices can hardly operate to best advantage if the wort is only allowed to cool to 140° F., since precipitation of the reversible protein-tannins does not commence in many cases until about that temperature. This provides one of the strongest arguments for pure air on coolers, since it is possible to remove much more of the fine sludge by cooling to 120° or 100° F. Such surface-active colloidal substances as proteins and hop resins contribute to the cooler sludge in considerable quantity. The particles tend to be adsorbed on precipitated particles or on the surface of the wort to form the skin, and are collected with the coarse sludge. Šatava[12] states that the coarse sludge of lager worts, some of which was precipitated in the copper and passed the hop strainer, contains about two-thirds protein and one-third hop resins and mineral matter. The "half fine sludge," amounting to about one-tenth the quantity of the coarse sludge, contains about 65% protein and 30% tannin, the remainder being carbohydrates and ash.

Heintz' view that neither hops nor barley husks contain true tannins was mentioned in Section 496. This is based on the finding that extracts did not yield the tannin colouration with ferric chloride nor give the alkaloid precipitate. It is contrary to the generally accepted view, based on acceptance of other criteria for substances that should be included in the tannin group. If 2 lb. per barrel of hops containing 2% of tannin yield the whole of this into wort, the latter would contain 0·12 gram per litre of tannin, without consideration of any derived from the malt husks. If such worts yield 0·3-0·4 gram per litre of dry sludge, the latter would contain 30% of tannin if all of it passed to the sludge. Actually some is not precipitated but remains in the cooled wort and beer. The wet sludge varies greatly in quantity and carries about 80% of its weight of wort, a source of considerable loss unless steps are taken to recover the wort. (Section 509.)

(506) Cold Aeration.

Pasteur found that the solubility of oxygen in wort of 1060 specific gravity was less than that in water, in the proportion of 1 : 0·862. According to Winkler's figures, a litre of water at 59° F. can absorb 7·04 ml. of oxygen from dry air. A litre of wort would, thus, absorb about 6 ml. The solubility is greatly reduced at higher temperatures, and hot wort at the refrigerators would contain very little oxygen. Any required for yeast growth must, therefore, be absorbed by "cold aeration" during refrigeration or subsequently. Though the quantity of oxygen that can be absorbed in this way is very small, only about 0·008 gram per litre, it appears to be essential for the initiation of a healthy brewery fermentation. Brown held that when yeast, in quantity such as is ordinarily used in brewing, was seeded into fully aerated wort it absorbed what he called the necessary "oxygen charge" in three or four hours. Yeast reproduction and nitrogen assimilation in aerated worts are about double that obtained in its absence. Whether the yeast makes direct use of the oxygen or depends on the oxidation-reduction potential of wort constituents does not matter here. The effect of air, if indirect, may operate through raising the potential to that required for yeast growth. The effect of the degree of air saturation of the wort on subsequent fermentation has been studied by Heron,[13] by a method which may be open to objections but appeared to give a reasonable explanation of results observed in practice. He found that the degree of air absorption varied from 10% to almost complete saturation with different types of cooling plant, and that defective fermentations resulted from lack of air or that frequent yeast changes then became necessary. Wort which was oxygenated to about 10% of saturation on a horizontal refrigerator, gained 5% at the fermenting vessel, but this could be increased to 87% by allowing a suction of air in the downfall main. Vertical refrigerators, 4 ft. 4 ins. in height, with the air drawn into the discharge pipe, gave 81 to 86% saturation, while this was 85% in the pan of a vertical refrigerator 4 ft. 9 ins. in height. With a 6 ft. refrigerator, in a room in which the air was changed once in six minutes, 95% saturation was obtained in the pan and complete saturation in the fermenting vessel. It was found that an appreciably harmful effect on the health, vigour and keeping properties of the yeast was caused if the saturation in the fermenting vessel was less than 85%, with the exception, perhaps, of fermentations on the Yorkshire stone square system, with which vigorous pumping is adopted. In general, it was found that oxygen deficiency could not be satisfactorily rectified by rousing in the fermenting vessel during fermentation, but good results were obtained when the worts were roused as they were run into the vessel, or within a few hours after collection.

It has not been found possible to measure directly the oxygen absorption in wort, nor have data been published regarding the effects of the oxidation-reduction potential of the wort on fermentation. Heron's results were based on determination of the oxygen saturation of water passed through the cooling system in the same manner as the worts, Winkler's method being used. The beneficial effects on fermentation must, therefore, not necessarily be ascribed to oxygen solution. Watkins' results appear to show that direct solution of oxygen, up to its very limited point of saturation, is very rapid and can take place almost instantaneously if wort is sprayed into a fermenting vessel. Very little solution of oxygen can occur above about 120° F., that is to say on an open cooler used in the ordinary manner, but there is no information at present on the effects of hot aeration on oxidation-reduction potential. All the practical observations so far published appear to show that oxygen requirements of yeast are only met by cold aeration. Thus, Watkins[14] found that 80% saturation, as measured with water, was necessary to ensure good and continuously good fermentations, using a Paraflow. Only 34·9% saturation was obtained at its outlet, when no method of aeration was used, the wort being delivered from a small wort receiver. After a 22 ft. fall to the fermenting vessels in a main supplied with an air inlet pipe, the saturation increased to 62·8%. With aeration at the hot end of the plate exchange refrigerator, 95% saturation was secured at the outlet and there was no increase on collection in the fermenting vessel.

The air requirements are complicated by the manner in which the wort is delivered to the fermenting vessels and should be studied in each individual case. If there is a fall with suction of air into the mains, no additional aeration may be required. If, on the other hand, there is a fall in the mains, which deliver into the bottom of the fermenting vessel, and no suction occurs, it may be necessary to admit air on the cold side of a Paraflow. If there is a rising main to the fermenting vessels and the wort is thereby delivered against a pressure of a few pounds, aeration at the hot end of the Paraflow may provide for both hot aeration and cold aeration. In other cases, all that may be necessary to provide sufficient cold aeration is delivery of the wort over the top of the fermenting vessel.

PROCESS OF COOLING

(507) Cooler Rooms and Open Coolers.

The "coolers" of English breweries or "coolships" of American are shallow vessels of great area, on which the nearly boiling wort is run to a depth of 6 inches or less to cool under atmospheric influence. Originally made of wood, they were a serious source of contact infection, as well as of contamination from the air, particularly when worts were exposed over-night for cooling. The worts frequently became acid or "foxed," as the darkening of colour accompanied by acidity was then called. The introduction of iron and copper in place of wood removed one source of contamination, but the risk of aerial infection remained when the coolers were, as is still frequently the case, erected in rooms surrounded by louvres or windows to admit as much air as possible and, with it, dust from the streets or adjoining premises or flies and pollen in the country. The extent of the risks was discussed in Section 384, but realisation that far greater danger resulted from condensation and drip from walls, beams and ceilings that had become breeding grounds of micro-organisms through the accumulation of saccharine matter was slow. In many old breweries the coolers are still a relic of days gone by. Surrounded by louvres, in a not too salubrious locality, and covered by a multitude of beams, little can be done, short of complete reconstruction, to make them safe. They must in any case be shut off from other parts of the brewery, particularly from such prolific sources of dust as malt mills and grains driers and there should be no right of way through the rooms.

In many modern breweries the coolers, refrigerators and air purifying plant are housed in special buildings, or the air inlet is placed as far away from any possible sources of infection as possible. No beams on which dust can accumulate or from which there can be any drip into the wort are tolerated. The roof and walls are domed or sloped at an angle of 45°, so that any condense will run down to suitably placed gutters in the floor. They are smoothly surfaced, so that they can be washed and kept in aseptic condition. In some instances, condensation has been prevented by cavity walls, heated by steam. They are evacuated through a cupola or by means of fans. There should be no access to the cooler when in use, observation being possible through large windows at the sides, while external controls are fitted.

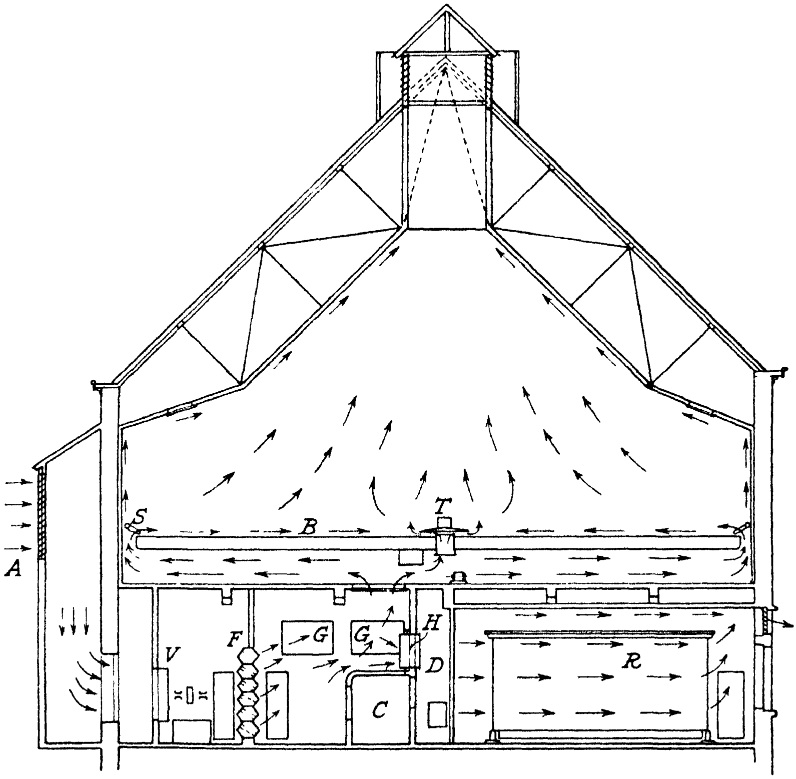


FIG. 119

COOLING INSTALLATION WITH PURE AIR (BRASSERIES ARTOIS, LOUVAIN)

Very elaborate air filtering and conditioning plant, generally serving both cooler and refrigerator room, have been installed where air purification is deemed desirable. Air temperature control is a great help in the prevention of condensation, but in every case it is necessary to find the most efficient combination of temperature and humidity by trial. In some cases 85°–95° F. is high enough, with a humidity of 40%, but it may prove necessary in others to heat the air to 105°–115° F. or reduce the humidity to 30%, or to combine high temperature and relatively low humidity. Diagrams of two new installations are given in Figs. 119 and 120, with photographs of the cooler represented by the former in Fig. 121 and another in Fig. 122.

In the installation illustrated by Figs. 119 and 121, a slight pressure of air is maintained by the fan (V), but in others, reliance is placed on the heat and cupola for evacuation of both cooler and refrigerator room. The air is passed from the inlet (A) through filters (F) across the refrigerators (R) and thence through openings in the ironwork to the cooler room above. Adjustable plates (S), operated from outside the room, are fitted around the cooler. These can be raised to direct the air current upwards and remove the steam, when at its maximum, or may be turned down, to direct the air current on to the wort and cause the necessary movement to break the surface pellicle. Finally, during settling and running off, the air is again directed upwards. The wort enters at the centre of the cooler over a revolving spreader, under which air can be aspirated, if desired. (H) is a radiator for heating the air, (D) the air distributing corridor for the refrigerators and (C) the control corridor.

The most striking feature of the installation shown in Fig. 120 is the provision of a copper dome over the coolship. The course taken by the air from the intake fan is clearly indicated. It passes through heaters into a filtered air plenum room, whence it can be distributed to the cooler or refrigerator room from which it is exhausted through overhead ducts, running the length of the cooler and refrigerator, vertically to the plane of the section. The domed roof of another cooler, with one of the two windows which form the ends of the room and the air dampers at the sides, are shown in Fig. 122. The photograph in Fig. 77 represents the cooler at a brewery where conditions are deemed to render air filtration unnecessary.

The coolers shown are constructed of copper or stainless steel, but sheet iron, cast iron and aluminium are also used. Iron has the great advantage of strength combined with low cost, but it corrodes rapidly and, like all other iron vessels used for wort or beer, coolers of this metal must be coated in some way to prevent rust and solution of the iron in wort. This is usually effected by mopping the cleaned and heated surface with a hot

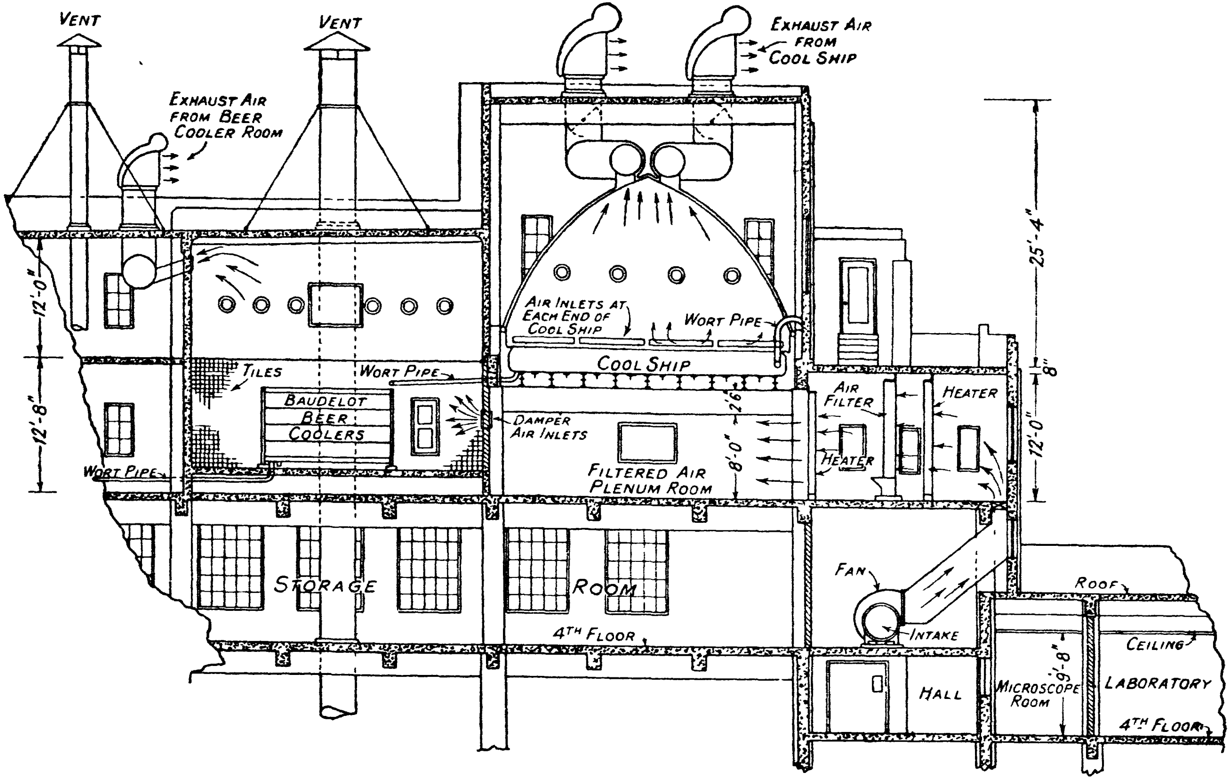


FIG. 120

SECTIONAL ELEVATION OF COOLING PLANT (ADOLPH COORS CO., GOLDEN, COLORADO solution of tannic acid, 1 lb. tannin in 1 gallon of water, which forms a hard surface of tannate of iron. This is preceded or followed by boiling a charge of spent hops and water in the vessel and the precaution taken to use it first for dark beers. Resistant enamels are also available for painting the iron. Copper is to be preferred to iron, as it is more resistant to wort, but aluminium is liable to warp on account of the irregular expansions on a shallow cooler. In any case the workmanship and finish is of as much importance as the metal itself. The plates must be perfectly joined, the slope regular and very slight, the position of the supports carefully determined. If these details are not attended to, the back will soon warp, forming pockets where wort will remain after running off, and fissures in which organisms can develop. Sedimentation is better, according as the depth of the wort is less. If this is about 6 inches, cooling is rapid and the surface great. Under such circumstances the complication of a float valve can be dispensed with, since sedimentation should be rapid and compact.

(508) Use of Coolers.

Practical experience has led to the conclusion that the movement for elimination of coolers was, to a certain extent, unfortunate. This applies particularly to lager breweries, in which the hop strainer is not such an efficient filter as the hop back of top fermentation breweries. Under such circumstances it forms an excellent settling back for coagulum from the copper, especially when there is any considerable proportion of fine sludge, as there may be with under-modified malts. This requirement is not so important when a hop back is used and brilliant wort is delivered, as is generally the case in top fermentation breweries, but many brewers consider that wort clarification is better, even then, than with the alternative, deep wort receivers. It may be supposed that the cooler wort on a shallow back ceaselessly descends, the hotter wort taking its place. The thinner the layer or greater the wort surface, the more active is this movement and larger particles, falling through the wort, attract and adsorb on their surfaces the extremely fine particles of the break.

(509) Cooler Grounds.

The amount of sedimentary matter deposited on the cooler varies greatly according to the amount of hot break passed through the hops and the time allowed for settling or treatment of the wort during cooling. It is also greater with badly modified malts or malts with high nitrogen content, and is generally less for dark than pale malts. Its dry weight may be as low as 0·05% of the weight of the malt if hop back filtration was effective but in other circumstances it may amount to 1½ to 3% of the malt. Brown gave figures for the deposit from a top fermentation brewery wort of 1076 sp. gr., containing 19·5% of solids and 0·135% of nitrogen ; 0·04% of the solids and 0·5% of the nitrogen was precipitated in a form containing 10% of nitrogen. Briant[15] found 1·30, 2·80 and 0·75 lb. of dry sludge from three brews of 100 barrels each. These last figures are trivial and nothing would be gained by endeavouring to separate the accompanying wort, but the higher weight given by Brown and the still greater quantities sometimes separated on coolers of lager breweries are quite a different matter. The wet sludge may carry with it some 3–5% of the wort cooled. This was formerly recovered by filtration through flannel bags, but this may take 10 or 12 hours and lead to much infection. Bags are, consequently, now replaced by filter presses by which the wort is recovered in 15–20 minutes. An example of a sludge filter is shown in Fig. 126. In many lager breweries the cooler is connected to a closed vessel, into which the sediment is flushed and from which it is forced by air pressure to a filter press. The filtered wort is boiled with the next brew or, alternatively, pasteurised for 25 minutes at 180°–190° F. and mixed with the following wort. In some instances it is fermented in a special vessel.

(510) Wort Receivers and Closed Coolers.

Closed or open iron or copper tanks, four to six feet deep, are used in the majority of British breweries to collect the bright wort from the hop back and deliver it by gravity to refrigerators. They should be enclosed and erected in rooms which are protected by well fitting doors from mill room dust or other sources of infection. It is claimed that they lack the advantages of open coolers and that these cannot be obtained on refrigerators. These objections lose much of their force with modern enclosed refrigerators, with which provision can be made for controlled hot and cold aeration, together with large cooling surfaces and provision for adaptation of the time of cooling through any range of temperature to suit individual worts. The advantages of space occupied and convenience, with abolition of the expensive air-conditioning installations, make it probable that the future is with receivers and aseptic refrigeration, rather than with open coolers and refrigerators.

In some breweries a compromise has been made by allowing the wort to stand for no longer than 20 minutes on an open

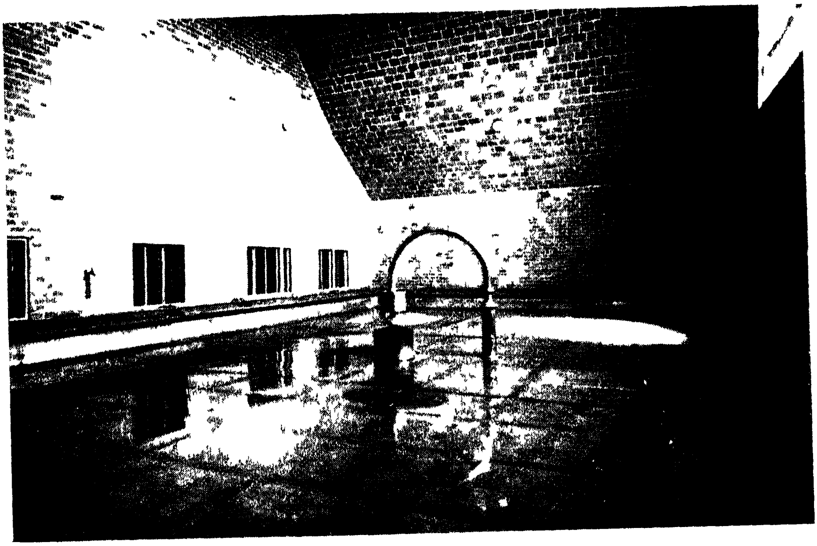


FIG. 121

AIR-CONDITIONED COOLER (BRASSERIES ARTOIS, LOUVAIN)

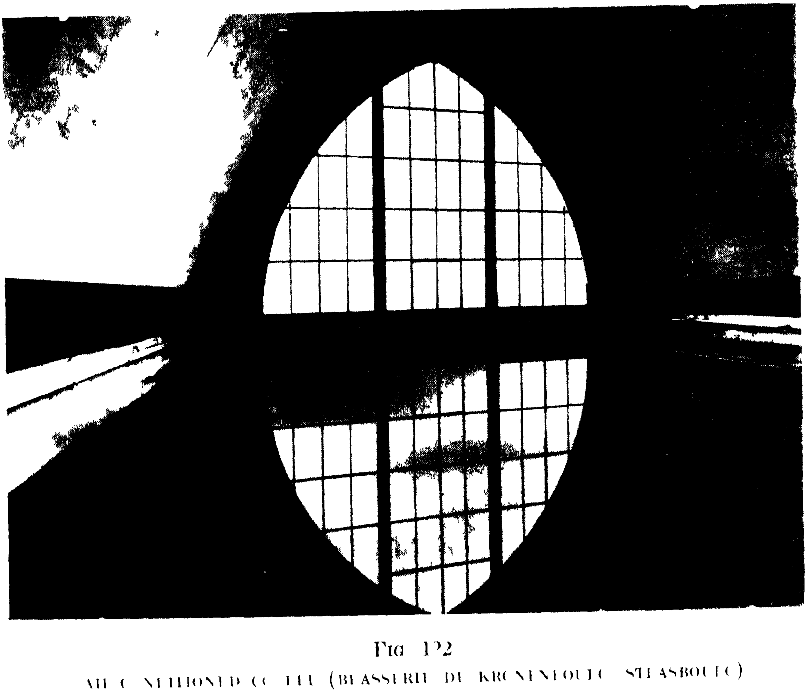


FIG. 122

AIR-CONDITIONED COOLER (BRASSERIE DE KRONENBOURG, STRASBOURG)

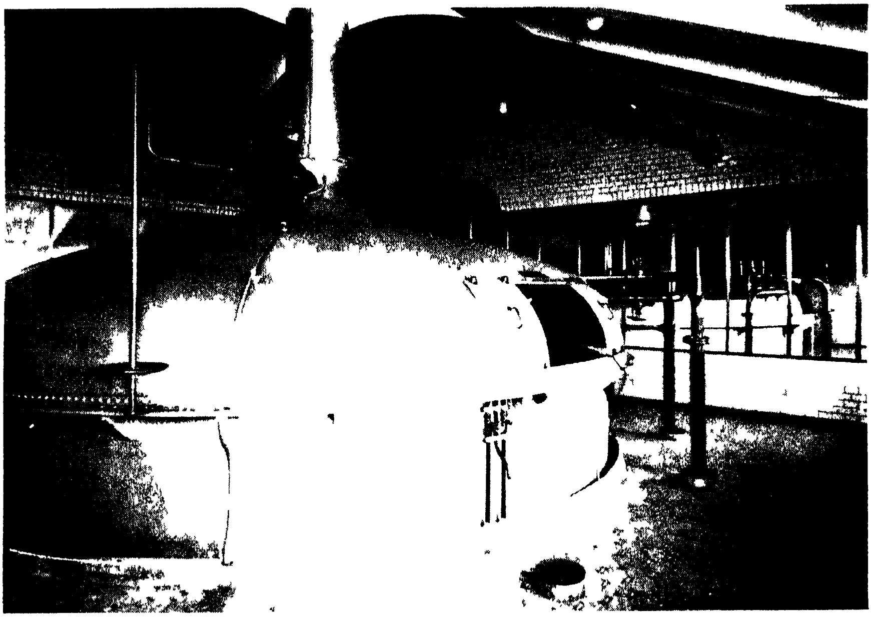


FIG. 123

CLOSED COOLER (ST. ERIK'S BREWERY, STOCKHOLM)

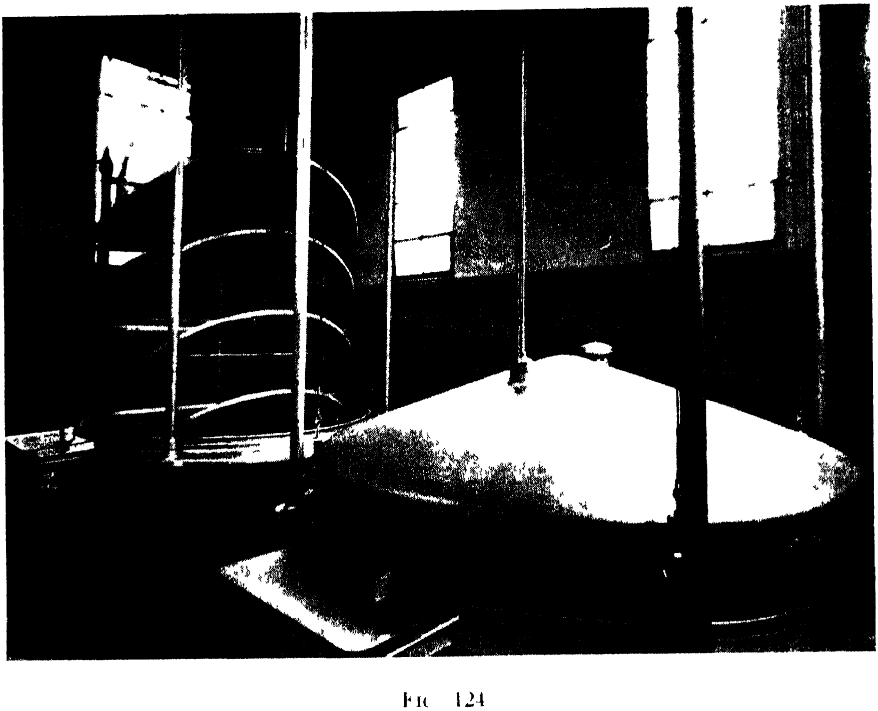


FIG. 124

NATHAN SEDIMENTATION VESSEL

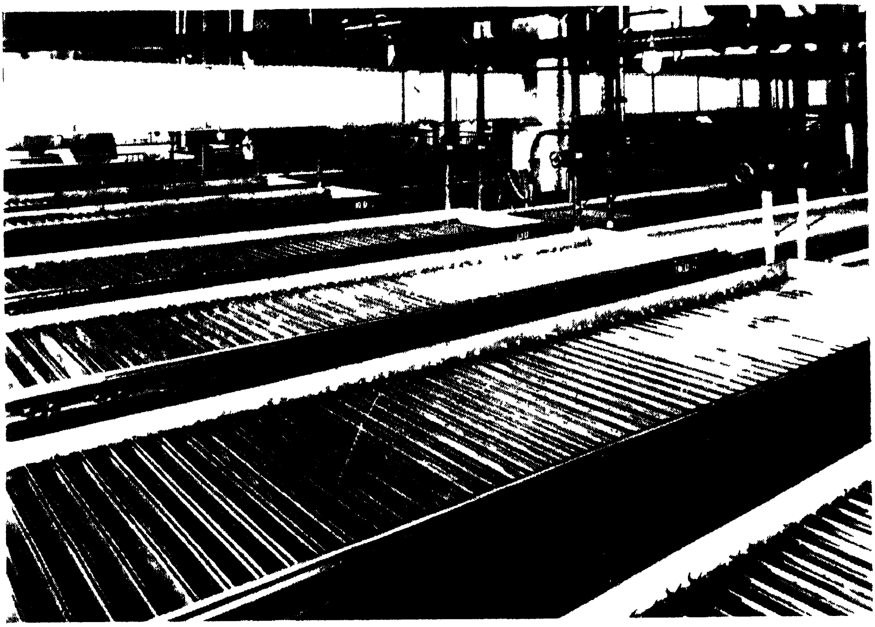
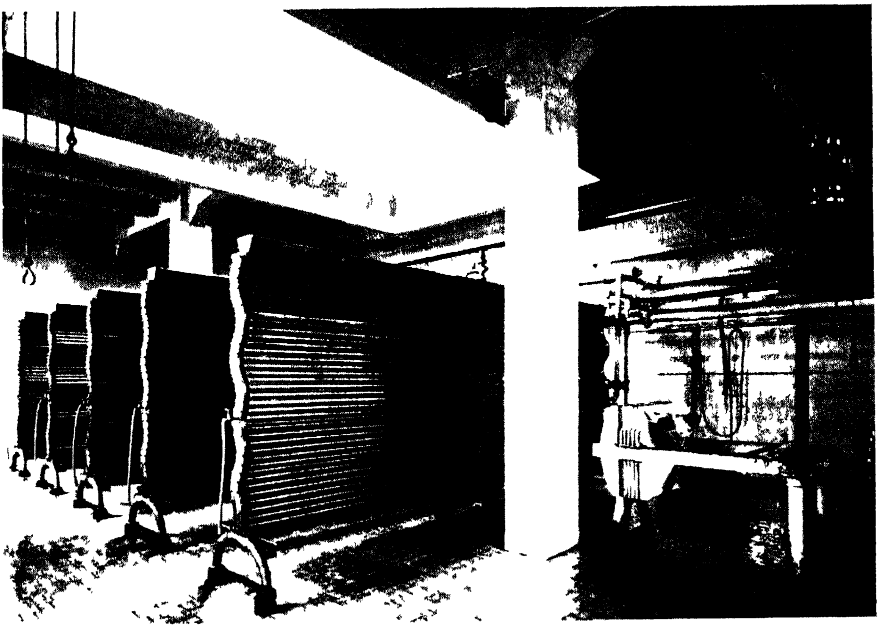


FIG. 125

HORIZONTAL REFRIGERATORS (ARTHUR GUINNESS SON AND CO. LTD. DUBLIN)



BAUDELOT REFRIGERATOR AND FILTER FOR COOLER GROUNDS
(BRASSERIES DE CHAMPIGNEULLES, FRANCE)

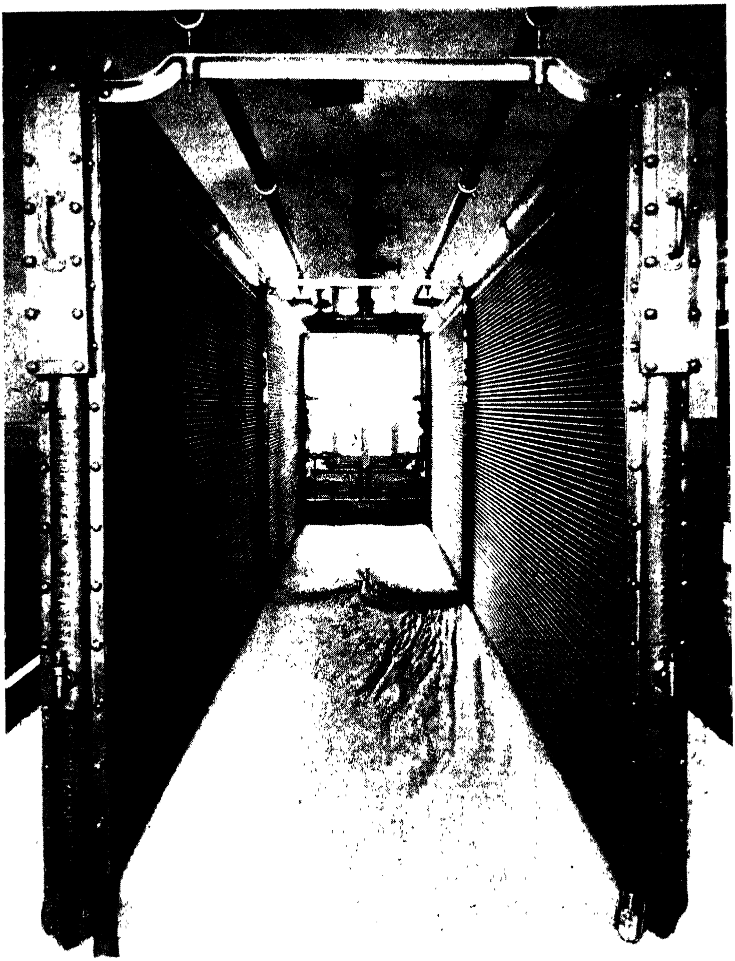


Fig. 127

VERTICAL REFRIGERATORS IN AIR-CONDITIONED ROOM
(BREWING CORPORATION OF AMERICA, CLEVELAND, OHIO)

cooler, from which it is run to a large, closed and sterilised settling tank where it is allowed to remain for about an hour for sedimentation, previous to refrigeration. In others, closed receivers have been fitted with cooling coils, Fig. 123. In some of these the temperature of the wort is brought down to between 125° and 120° F. in an hour and a half, while a constant stream of purified air is passed through the wort In other cases the refrigerator is dispensed with and the worts are completely and aseptically cooled to fermentation temperature in the tank, brine coils being installed if necessary. Plant of this type has the advantage over coolers of great economy of space, but sedimentation and flocculation are not generally so satisfactory as with coolers or wort receivers and refrigerators.

(511) Nathan Sedimentation Vessel.

A device consisting of a series of superimposed aluminium plates in a closed vessel is adopted in the Nathan Brewing System to facilitate deposition and retention of sludge from the cooling wort and to facilitate the delivery of sterile wort to the fermenting vessels. In this way certain advantages of the open cooler are retained without exposure of the wort. The plant, with plates withdrawn for cleaning, is shown in Fig. 124. The hop strainer in the Nathan system is closed and insulated, the wort is passed through it as rapidly as possible, and the mains to the sedimentation vessel are also insulated and as short as possible. Almost boiling wort consequently reaches the settling tank and sterilises the latter. This vessel is constructed of aluminium, is covered with a water-sealed dome and insulated with cork in order that the wort, which is pumped from it to a vertical refrigerator in a germ-free room, should reach the refrigerator as hot as possible. This is found to improve flocculation and sedimentation. The wort refrigerated to between 34°–39° F. is returned to the bottom of the sedimentation vessel. Hot wort is continually pumped from the top of the latter through a float valve until the whole bulk is cooled to pitching temperature. Very little mixing occurs and 70 barrels can be cooled in 1½ to 2 hours. Sufficient aeration is secured by blowing 30,000 cubic feet of filtered air per hour through the refrigerator room. The particles of hot break carried through the hop strainer and the coarser precipitate formed during cooling adsorb the finer break and cause it to deposit firmly on the large surface presented by the plates. The sedimentation can be improved by moving the plates, so that the deposit first formed slides off and mixes with the wort. It then adsorbs more fine break, with which it again settles rapidly.

Sufficient time for sedimentation is allowed to secure a clear wort. The general objection to this is recognised but its force is contested by the originator of the Nathan system, who claimed that the suspended matter is foreign to the wort and that its removal as completely as possible is a great advantage.[16] By complete clarification a slower fermentation and reduced yeast reproduction may result, but the yeast remains cleaner and does not require washing. He also held that all substances deposited during boiling and cooling should be removed to prevent detrimental effects on the flavour of the beer.

(512) The Wooldridge System.

The aim of the originator of this process was to secure delivery of a sterile wort at the fermenting vessel by boiling in a closed copper, followed by cooling, partly in the same vessel and partly by means of a closed counter-current refrigerator. The wort is run from the mash tun into a pressure boiling vessel heated by steam coils and fitted with a false bottom to retain the hops and, in some cases, with a cooling coil. The wort is raised to a temperature of 228° F., under a pressure of 5 lb. per square inch, by means of steam at 30 lb. pressure. The valve of the vessel is slightly opened, in order that there may be ebullition with no more than a slight fall in pressure, and boiling is continued for half an hour. Steam is then shut off and the pressure released, the temperature of the wort falling to its normal boiling point. The pressure above the wort is then gradually reduced to 4 inches of mercury with a vacuum pump. The temperature of the wort rapidly falls to 126° F., the reduction being accelerated by water circulation in the coil. The boiling point is more rapidly reduced with the fall of pressure than the temperature of the wort, which consequently remains in violent ebullition. This is held to be an important point in the process. When the temperature has fallen to about 126° F., the wort is circulated from beneath the false bottom, through the counter-current chiller and back to the boiling vessel by means of a rotary pump, until it has all reached the pitching temperature. A short time is then allowed for the hops to settle. The wort is run through them and the false bottom to the fermenting vessel. This system has been adopted in some top fermentation breweries, the claim being made that flocculation is very good and that a bright sterile wort is obtained. The wort is cooled from its boiling point to 58° F. in 2½ hours, with 300 barrels of cooling liquor at 55° F. for 200 barrels of wort.

WORT REFRIGERATION

(513) Horizontal Refrigerators.

The earliest type of refrigerator was an adaptation of the open cooler, in which coils for water circulation were laid to increase the rate of cooling. From this, improved horizontal refrigerators were developed in which the hot wort flowed gently between vertically disposed flattened tubes, spaced about 4 inches apart, throughout the length of a shallow copper pan. These refrigerators have the disadvantage of occupying a large floor space, but it has been claimed that they materially assist in wort clarification and, in conjunction with vertical refrigerators, they form a powerful cooling arrangement which is economical in water requirements. Wort at 190° Fahr., run first over a vertical refrigerator and then over a horizontal, with liquor at about 50° to 55°, may be cooled to 60° with one barrel of liquor for each barrel of wort.

(514) Vertical Refrigerators.

Vertical surface refrigerators have almost completely superseded horizontal types on account of their greater convenience and simplicity in cleaning, combined with efficiency. They vary considerably in details of construction, but among the essential points are that the wort should flow over the whole surface in an even and thin stream and that the interior of the liquor pipes should be readily accessible for cleaning, as the accumulation of scale rapidly and materially reduces the cooling effect and may prevent the possible use of the heated liquor for mashing. The construction must be robust and all joints made in such a manner as to resist cleaning with powders or dilute sulphuric acid and yeast. The size and shape of the pipes should be designed for economy in cooling liquor and strength and in such a way that there are no places in which wort solids can collect and remain inaccessible to cleaning.

The Baudelot was an early type of vertical refrigerator which is still very largely used, particularly in lager breweries. It usually consists of a series of 36 to 48 pipes of circular section and about 2⅜ inches diameter, between vertical supports and connected by U bends for circulation of the cooling liquor. In newer types, Fig. 126, the pipes have a serpentine arrangement to increase the surface, and a flat strip of metal may be attached underneath each to convey the wort on to the next pipe. The pipes are completely separated one from another so that they can be readily cleaned. A section, about one-third the height of the refrigerator, is reserved for brine circulation or direct ammonia expansion, iron pipes being used for the latter instead of copper. In some cases dilute alcohol is used as the refrigerated liquor. The capacity of a 48-tube refrigerator is governed by its length. It should cool 5 barrels of wort per hour per foot of tube length, using one to one and a half barrel of water per barrel of wort cooled to 70° Fahr. when it reaches the ammonia pipes. The wort runs into a copper trough at the foot of the refrigerator from which it is delivered to the fermenting vessels.

The flat tube type of refrigerator, developed in England and of which examples are shown in Figs. 127 and 128, is more efficient than the Baudelot, and consequently occupies considerably less space for an equal output. The tubes are flattened and connected at either end alternately by vertical members, which are easily removed for cleaning. The wort flows in an evenly distributed stream from a perforated main into a trough which may be perforated or made without holes, to avoid the clogging caused by hop seeds; the wort flowing over it is distributed in a thin film over the pipes and thence to the bottom trough. A refrigerator of this type, 6 ft. 3 in. in height, by 12 ft. 4 in. in length, overall dimensions, with cooling surface 10 ft. by 5 ft. 8 in. is capable of dealing with 50 barrels of wort an hour with cooling liquor at 52° Fahr. It is advisable to have ample cooling surface and run the wort slowly in a germ-free atmosphere, for which reason a refrigerator about 8 feet high is generally more satisfactory. If spraying occurs, the wort is running too fast or the grooves between the tubes are too narrow to permit of even flow over them. The amount of cooling liquor required varies greatly with its temperature, but usually about 2 barrels at 52° Fahr. are required to cool 1 barrel of wort from 180° to 60° Fahr.

(515) Refrigerator Room.

Refrigerators should be installed in closed rooms, supplied with filtered air, which is, with advantage, warmed to minimise steam and evacuated by means of a cupola or fan. All controls should be outside the room, with thermometers placed in such a position that they can be seen from outside. Ample space must be allowed for cleaning, with an impermeable floor, properly laid for drainage and all angles rounded. The walls are tiled or surfaced with impermeable, washable materials. The ceilings must be constructed entirely without beams, to avoid drip, and preferably vaulted to ensure good ventilation. Condensation is minimised if the bottom plates of the cooler form the ceiling of the room, or other means of heating the roof are adopted.

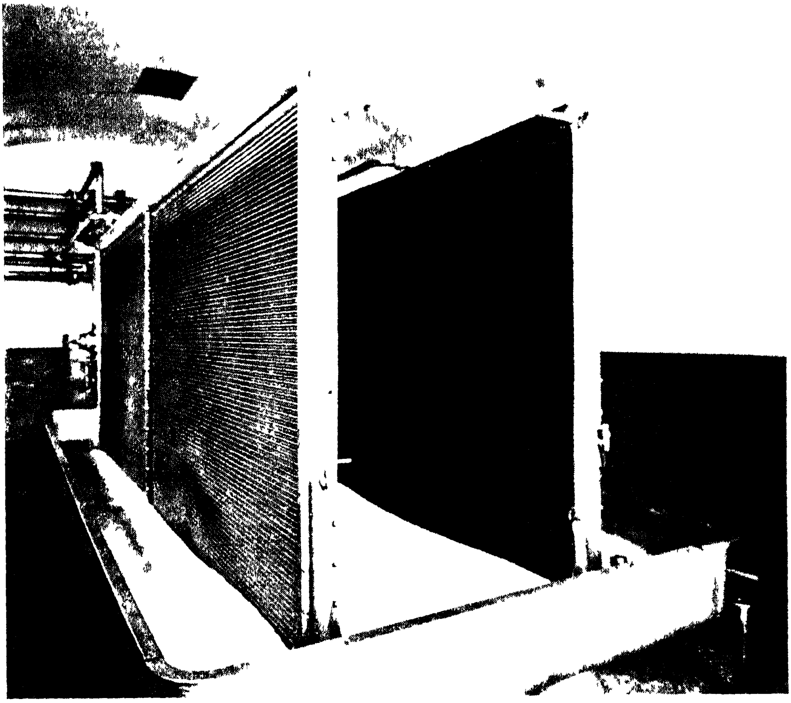


FIG. 128

AIR CONDITIONED REFRIGERATOR ROOM
(SCHREIBER BREWING CO., BUFFALO, N.Y.)
(DETAILS OF INSTALLATION IN FIGS. 78-80)

To face p. 276

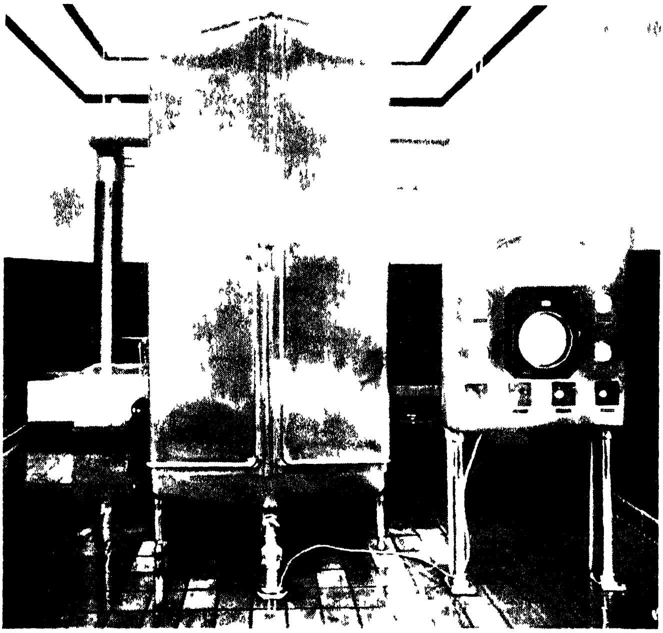


FIG. 129

MOJONNIER WORT COOLER FOR 225 AMERICAN BARRELS PER HOUR
THE AIR CONDITIONING UNIT IS ON THE FLOOR TO LEFT
(MINNEAPOLIS BREWING COMPANY, MINNEAPOLIS)

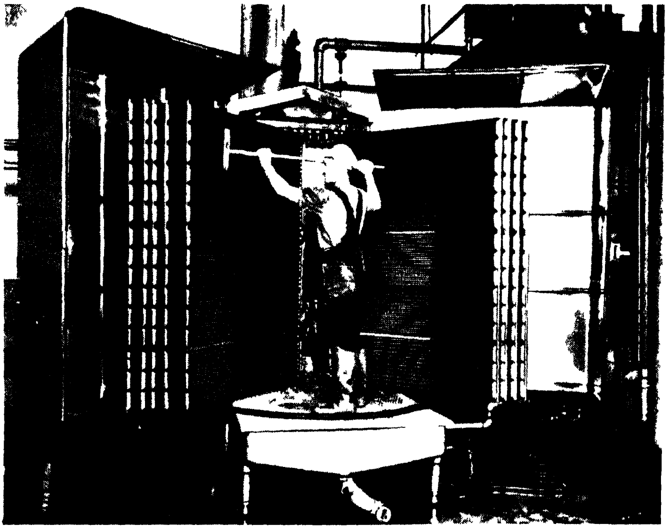


FIG. 130

MOJONNIER WORT COOLER IN OPEN ROOM
(GLUEK BREWING COMPANY, MINNEAPOLIS)

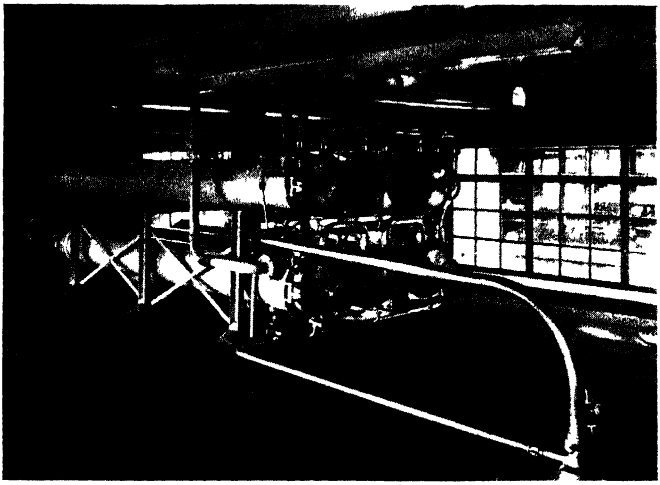


Fig. 131

SULZER COUNTERFLOW COOLER (BRAUEREI HALDENGUT, WINTERTHUR)

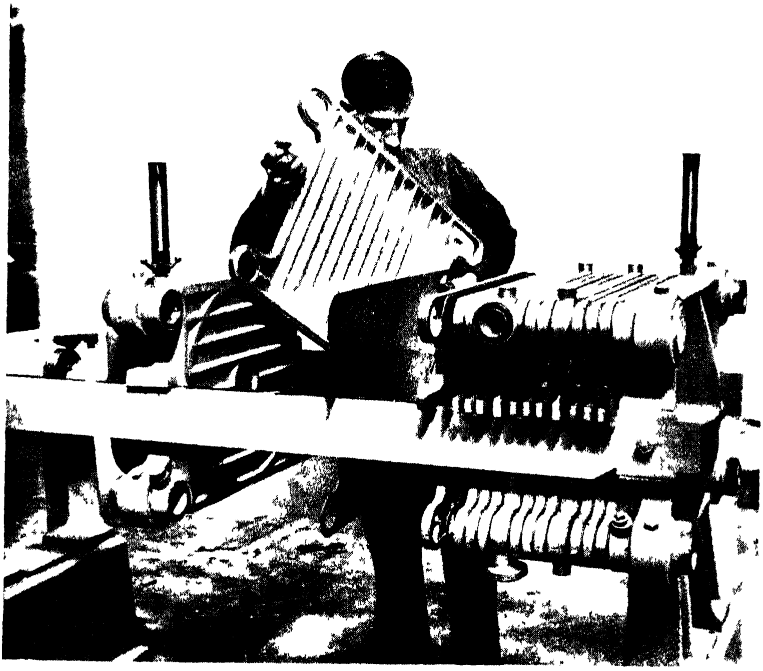


Fig. 132

PARAFLOW PLATE EXCHANGE REFRIGERATOR

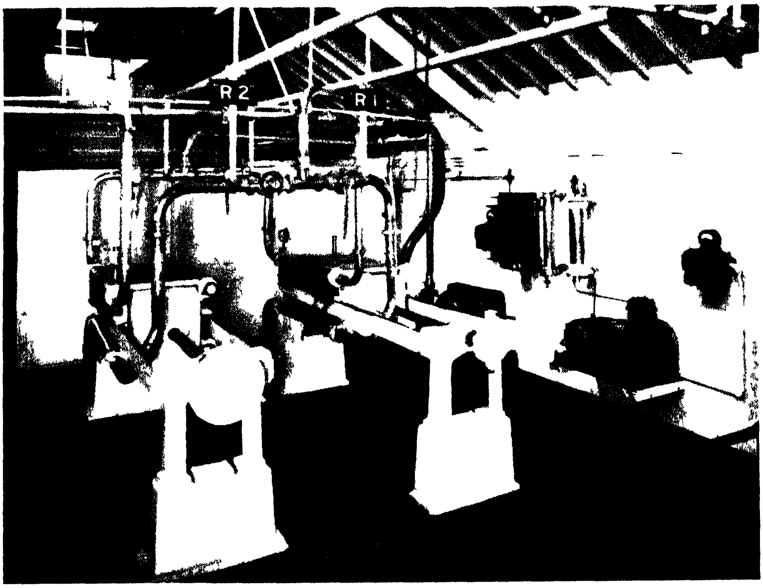


FIG. 153

TWO REFRIGERATORS IN PARALLEL SHOWING THE [illegible] [illegible]

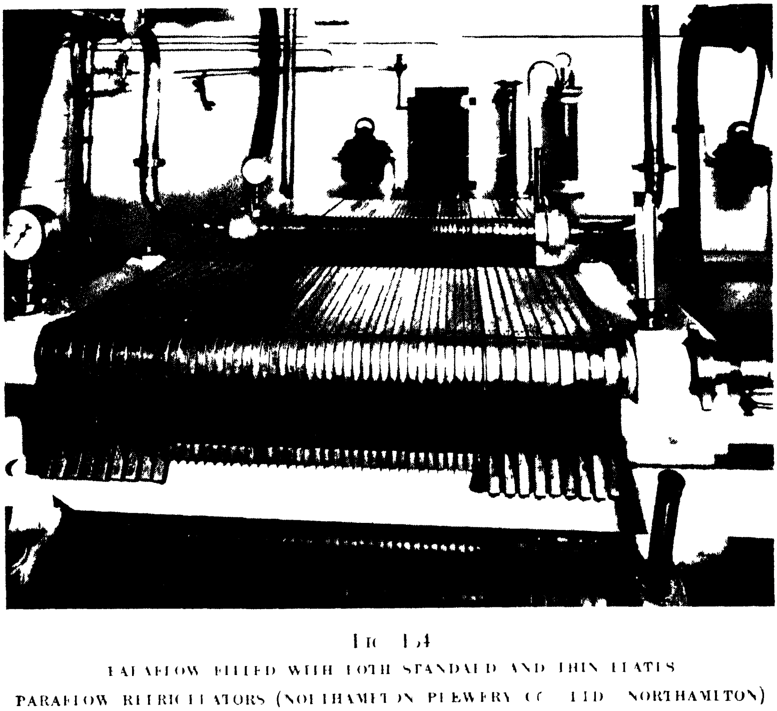


FIG. 154

PARAFLOW FITTED WITH BOTH STANDARD AND THIN PLATES

PARAFLOW REFRIGERATORS (NORTHAMPTON BREWERY CO. LTD. NORTHAMPTON)

Various important points in the design of enclosed refrigerator rooms and operation of an air conditioning and filtering unit, originally described by H. R. Henius, were illustrated in Figs. 78 to 80. The windows for observation on one side of the room consist of two pieces of plate glass, mounted in aluminium frames. The glass is tightly sealed on the inside, but there are two small slots to allow circulation of air between the glasses and prevent accumulation of moisture. The walls and ceiling are finished with Keen cement, covered with an undercoat of paint that never dries flint hard and finished with glossy enamel. This has proved superior to tiles or terra cotta, besides being cheaper in original cost and upkeep.

(516) Enclosed Refrigerators.

Attempts to enclose the vertical refrigerators were not very successful, prior to the introduction of conditioned air, but this method of preventing aerial infection is now a practical proposition, as exemplified by the Mojonnier wort cooler shown in Figs. 129 and 130. This is very compact and consists of a number of stainless steel cooling plates, housed in a stainless steel casing. The upper tubes of the sections can be cooled with water and the lower with ammonia or brine. Filtered and heated air is continuously circulated, at the rate of one change per minute, through the housing when the refrigerator is in operation. The conditioning unit is placed close to the cooler, and all gauges, thermometers and switches are mounted on an instrument board. Wort flows over the individual cooling sections from a distributing trough and each section is joined to the refrigerant supply by swivel connections to facilitate cleaning, as shown in Fig. 130. It is, of course, quite unnecessary to install the refrigerator in an elaborate room, such as that shown.

The counter-current cooler, so largely used for rapid chilling of beer for bottling, has not received general acceptance as a wort cooler, on account of the difficulties of inspection and of effectively removing the sticky deposit from long tubes. The Sulzer counterflow cooler, Fig. 131, is an efficient type that has found successful application in wort cooling. It consists of six cylinders arranged in series, each containing 18 copper tubes of 2-inch internal diameter and 13 feet long, also arranged in series in sets of three. The cooling liquor flows outside the tubes in the opposite direction to the wort. Water can be used in some of the cylinders and brine in others, their covers being removable for cleaning.

(517) Plate Exchange Refrigerators.

The principle of a plate heat exchanger was adopted by R. Seligman in the design of the Paraflow refrigerator. This consists of a series of cast gunmetal plates, with grooves on each side, mounted alternately with smooth copper plates in a rigid frame and screwed tightly together, Fig. 132. The grooves provide what is, in effect, two continuous series of flattened pipes, connection from the portion on one plate to that on another being made through circular apertures at the corners of the plates. Wort flows up and down one side of each plate, and water, in the reverse direction, along the grooves on the other side, which is separated from the next wort grooves by the thin copper plate. The length of the flattened pipe on each side of a standard plate is about 20 feet, giving a total length of travel of 140 feet in a machine with 7 plates. Such a machine can take about 10 barrels an hour, the wort and liquor being pumped through by centrifugal pumps, the rate of travel being 375 feet per minute, with a high rate of heat transmission. It is not practicable greatly to increase the rate of flow, since the pressure required increases more than proportionately. The device adopted is to run two plates in parallel for 20 barrels or four for 40 barrels an hour, as shown in the diagram, Fig. 135, on which the wort temperature at various points is also given.

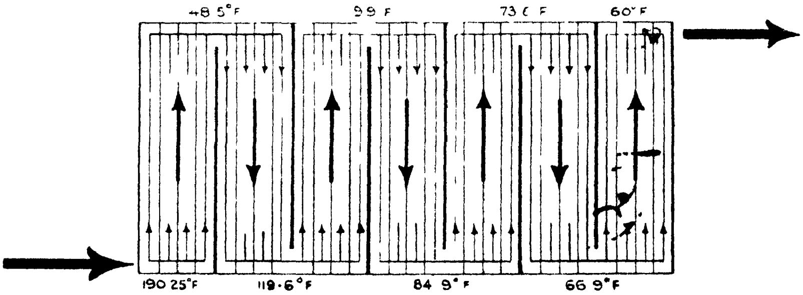


Fig. 135

FLOW DIAGRAM OF WORT AND WATER IN PARAFLOW

Watkins[18] noted that the wort was cooled from 120° to 80°, while passing the 3rd, 4th and part of the 5th time through the machine, with excellent flocculation. The results of the experiments on rapid cooling, described in Section 503, were so striking that a new design of plate has been introduced to permit of a great reduction in the time required to cool through a selected temperature range when this is required. The grooves on the new plates are wider than those on the old, but much shallower. They can be substituted for the deeper plates in any section of the machine and thus give added control over the time of cooling through any range selected to suit a particular wort. Ten sets of thin plates for 5 barrels an hour each, substituted for the 2nd, 3rd, 4th, and 5th of the total seven passes in a 50 barrel an hour machine, reduced the time required to cool from 155°–150° to 74°–73° from 15 seconds to 4 seconds. As a result flocculation was more rapid in the particular wort cooled, and this made it possible to skim a sludge head off the fermenting vessel earlier than before. The Paraflow refrigerators can be installed in many different ways according to requirements. They can be used singly up to 60 or 80 barrels an hour. It is usually preferable to run smaller units either in parallel or tandem for larger outputs. In the example given in Figs. 133 and 134 both wort and liquor are divided between two machines and reunited in a common main after cooling. Aeration is usually effected by a small air pump delivering purified air into the inlet main, where the temperature is usually sufficiently high to ensure sterilisation. This secures adequate hot and cold aeration in most cases, but in some it is necessary to admit sterilised air into the outlet main to get satisfactory aeration. Among the advantages of this type of refrigerator are its compactness and suitability for placing in any convenient position, since no protection from infection is required. The degree of aeration can be controlled and cleanliness assured, both in wort and liquor circuits. The very large surface ensures good break and sedimentation. Before use it is sterilised by steam or boiling water under pressure of the pump.

Fig. 133 is from a photograph of a Paraflow refrigerator installation with a capacity of 160 barrels an hour, showing the air compressor and electrical air sterilising plant. Fig. 134 is a close-up of the same refrigerator, showing the standard plates at either end and narrow plates at the centre for speeding up the temperature drop, in this case between 155° and 80° F.

The plate exchange system can equally well be applied to heating as well as cooling, so that it becomes a very efficient sterilising system. The two methods of working can be combined if it is desired to make the fullest use of an open cooler and by cooling wort to about 80° F. obtain the greatest possible precipitation. The wort enters the sterilising plant at this temperature, where it is heated by boiling water to between 176° and 196° F. and cooled down again to between 120° and 130° by the incoming wort before it is passed to the refrigerating unit from which, with the necessary care, sterile wort can be delivered to the fermenting vessels. Cooler grounds filtrates can be sterilised and cooled in a similar manner.

(518) Cooling Liquor.

Certain of the elementary principles of heat transference applied in wort cooling may here be appropriately referred to in connection with the relative efficiency of various types of refrigerators. Heat transfer through metals is very rapid, but a stationary film of water 3/1000 inch thick is equivalent in resisting heat flow to a copper plate $2\frac{1}{4}$ inches thick (Heastie[19]). Stationary wort on a cooler or in a pipe therefore cools very slowly. In a pipe there is always a film of smoothly flowing liquid against the wall, through which heat must be transmitted. The thickness of this film depends on the viscosity of the liquid. For efficient heat transfer in a counter-current cooler, it is necessary that the hot liquid in the inner tube and the surrounding cooling liquid both move at the highest possible speed. Confirmation of this may be obtained from fermenting vessel attemperators, in which a rapid flow of water is equally as important as a low temperature.

On a vertical refrigerator 6 feet high, the rate of fall of the wort is only about 51 feet per minute, so that there can be no high rate of heat transmission. It has been found that the rate of transmission on a refrigerator, including the loss of heat to the atmosphere, was about 200 B.T.U. per square foot per degree F. mean temperature difference when the water ways have been newly cleaned, falling to about 150 B.T.U. three months later (Seligman[20]). The loss of efficiency may be much greater than this, when scale is formed from very hard waters or there is any marked growth of fungi. Double-pipe counter-current coolers are more efficient, as a high speed can be obtained in both liquids, but the circular pipe commonly used cannot give the highest rate of heat exchange. Tubes of narrow flat cross-section, such as are used on modern refrigerators, are much more efficient and they can be conveniently secured by adoption of the plate exchange system of heat transfer.

Reference has already been made to economy of liquor with vertical and horizontal refrigerators in tandem. This is only an example of the principle that any well designed refrigerator has a low liquor consumption when working at a low rate, with large cooling surface, in comparison with the amount of wort cooled, provided heat transfer is unimpeded by dirt or scale. The consumption of cooling liquor depends on the difference of temperature between the liquids, on the surfaces of contact of the hot and cold liquids with the separating metal, on their speed of flow and on the cleanness of the heat exchanging surfaces. A vertical refrigerator of the type commonly used in

England should cool a wort through 120° Fahr. to 60° or within 5° of the liquor, using 2 barrels of water for each barrel of wort. In order to cut this consumption down to half, it would be necessary to increase the cooling surface five-fold, or reduce the temperature of the liquor from 55° to 45° Fahr. In the former case the liquid would be heated to 115°, in the second to 165°. The Paraflow is subject to exactly the same rules as any other efficient refrigerator.

The heated liquor may be used for mashing, if its purity is assured, but it is more frequently employed for cask washing and similar purposes.

Such an increase of surface as that required to cut down the liquor consumption by half, when its temperature is about 53° F., is usually impracticable, and it is frequently necessary to chill liquor during the summer. A chilling unit, with automatically controlled ammonia supply, can be partly seen in Fig. 80. The pipes and valves, visible through the window, constitute the automatic control. The unit for 105 British barrels per hour is 20 feet long, 6 pipes high and 6 wide, with a total length of 1,106 feet of 1¼ inch pipe. It cools 97 Imperial gallons of water per minute, from 60° to 35° Fahr., with an insulated galvanised iron tank for 22 barrels of water. The liquor is pumped from the chilling unit, through the refrigerator and returned to the top of the former. It is constantly circulated during cooling and seldom requires replenishment. An ejector-compressor type of refrigerating machine[21] is also successfully used for the same purpose.

(519) Summary.

Wort cooling would be a simple operation were it not that important changes in the physical state of some of its constituents were involved, and an opportunity presented for microbiological infection. These points have been borne in mind in all recent improvements in plant. The danger of infection was first realised and many brewers took the very drastic step of abolishing their open coolers. Closed coolers of various types, and refrigerators which increased the rate of cooling were introduced. This was soon found to involve difficulties in clarification, which were not understood until the significance of the cold break was more fully appreciated, and the nature of the wort constituents concerned was discovered. It took many years to find the conditions most favourable to cold break and removal of the protein-tannins. Many factors appeared to be involved. These were gradually detected. Their individual significance was probably over-rated at the time, and appropriate modifications of plant were made with each discovery. Among the advantages of the open cooler, is the opportunity afforded for adsorption of finely divided colloidal matter or the depositing hot break. This finely divided precipitate is, however, formed most abundantly at temperatures below those regarded as safe for wort exposed in open vessels to aerial infection. The difficulty is to make it aggregate into flocks, which are readily removed during refrigeration or the early stages of fermentation.

It was thought at first that flocculation was promoted by agitation during cooling. Then the advantages of long travel and immense surface exposure or contact with the containing material were insisted on. Finally the significance of the rate of cooling from 140° to 70° F. was observed. No finality has been reached, but it is clear that worts are not consistent in their requirements. Some break best if very rapidly cooled. Others need longer time, and some refuse to break at all, but this defect can usually be traced to unsatisfactory materials or boiling. Plant is available for putting all these factors into operation, but further developments will no doubt be required, as more is learnt about the nature of the precipitated material and the conditions favouring its flocculation.

Aerial infection is no longer regarded as the chief source of biological difficulties. Dirty plant, drip from infected beams and ceilings, and the invasion of microbe-carrying flies are much more to be dreaded. Safety demands complete demolition of many old cooling rooms and reconstruction of the majority. Air purification has found one of its most useful applications in cooler and refrigerator rooms, but should be combined with warming to a sufficient degree to abolish condensation of steam and drip. The great fight now is between the upholders of free exposure of wort to pure air under aseptic conditions, and those who advocate some form of enclosed refrigerator, which shall comply with all the requirements of colloidal flocculation, while avoiding the cost of air conditioning plant and specially constructed rooms.

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CHAPTER XXX

BREWING ROOM CALCULATIONS AND BRITISH EXCISE REGULATIONS

(520) Materials for a Brew.

The brewing list, or programme of brews drawn up weekly after a survey of sales, stock and forecast of requirements, is complicated in many breweries by the number of qualities produced and the necessity of spacing those from which pitching yeast is obtained, so that this shall be available of correct age when needed. Stocks of materials are checked and arrangements made for vessels and liquor treatment. Calculation of the quantities of materials for each brew is based on their analyses, with an allowance of 1 or 2% for extract loss. The extract required for 340 barrels of beer of original gravity 1045·5 is, for example,

$$340 \times 45{\cdot}5 = 15{,}470 \text{ degrees or } 5{,}569 \text{ brewers' pounds.}$$

Suitable materials, when 85% of the extract is to be obtained from malt and 15% from sugar, would be 50 quarters of malt with an average extract of 97 lb. and 24 cwt. of sugar, extract 72 lb. per quarter of 2 cwt.

$$\begin{array}{lrlr}
50 \times 97 = & 4{,}850 \text{ lb.} & \text{or } 50 \times 269{\cdot}4 = & 13{,}470 \text{ degrees} \\
12 \times 72 = & 864 \text{ ,,} & \text{or } 12 \times 200{\cdot}0 = & 2{,}400 \text{ ,,} \\
\hline
 & 5{,}714 \text{ ,,} & & 15{,}870 \text{ ,,}
\end{array}$$

The details of the grist would then be worked out in the manner outlined in Sections 394–398.

This particular brew and the following examples, illustrating the make-up of copper lengths and parti-gyles, are based on wort A of Table 198, Section 450. This could have been obtained from 50 quarters of malt, yielding 13,298 degrees or 4,788 lb. of extract which, with 24 cwt. of sugar, gives a total yield of 15,698 degrees or 5,652 lb. Hops have been calculated at the rate of 7·2 lb. per quarter of materials, to give a round figure of 4 cwt. for the brew.

(521) Losses from Mash Tun to Fermenting Vessel.

Before proceeding to the calculation of copper lengths required to give 340 barrels in the fermenting vessels, it will be well to examine the nature of the losses which may be anticipated. These consist of volume shrinkages and actual losses of extract, which vary considerably with different plant. The figures given can, therefore, only indicate the nature of the allowances which have to be made when calculating the copper lengths or quantity of liquor required for a brew.

Extract Losses. (1) In grains, through incomplete conversion and retention of wort. These should not amount to more than 1 or 2%, and can be reduced almost to nil, with well modified malt, by accurate weighing, efficient milling and mashing. The yield in such circumstances is not infrequently greater than the laboratory extract.

(2) Absorption of wort in spent hops. This is largely, but never completely recovered in the hop sparge. When the worts are divided in two or more coppers, at different gravities, the strong wort retained by the first copper hops is displaced by weaker worts of later coppers turned over the hops in the hop back. The weak worts are then more or less completely washed out by an efficient sparge of about 6 barrels per cwt. of hops.

(3) Loss in plant. 1% should cover the loss of extract due to adhesion of wort to plant.

Shrinkage of Volume. (1) Absorption in grains, about 92% of weight of malt mashed, or 0·86 barrel per quarter.

(2) Absorption by spent hops in hop back, about 6 times their weight or 2 barrels per cwt.

(3) Evaporation during boiling. In open coppers should be about 8–10% per hour. Reduced to about 3 barrels per hour in pressure coppers.

(4) Evaporation during cooling. May be about 5% on open coolers and refrigerators. Much reduced with wort receivers and enclosed refrigerators.

(5) Shrinkage of volume on cooling. This amounts to about 4% between 212° and 60° F., but does not represent a loss of liquor.

(6) Small loss of about 1% by adhesion to plant.

The shrinkage of volume during cooling has to be taken into account when making up the copper lengths, because the volume of wort in the copper is ascertained at boiling point and that of the wort collected in the fermenting vessels at about 60°. The shrinkage amounts to about 4·2%, but an addition of about 0·3% has to be made for expansion of the copper, to correct for its gauging at ordinary temperature. Hence the approximate allowance of 4% for volume contraction between 212° and 60° F. These losses and volume changes are particularised in Table 217 for wort A of Table 193, assuming that the given lengths were measured in the copper at 212° F.

At this point it is necessary to decide whether the copper length should be increased by addition of liquor. This length, known as "copper in," must be made up with due consideration of the length and gravity of the wort to be collected in the fermenting vessels. It will be noted that the extract from the mash tun is sufficient to produce about 220 barrels at 1060 sp. gr. This length could reasonably be expected from 295 barrels of mash tun worts, allowing for evaporation during boiling and shrinkage on cooling. If a stronger all-malt beer is required, it would be necessary to divide the mash tun worts into two or more copper lengths of stronger and weaker worts. The strong beer would be made from the first copper, its gravity being adjusted on collection in the fermenting vessel by addition of a small proportion of the weaker worts. The weaker beer would be made from the later coppers, with the necessary proportion of strong wort to adjust its gravity. (Section 523.) Alternatively the later runnings from the mash tun are held over for use with the mashing liquor of the next brew.

Liquor can be added to the copper for weaker beers. It will be noted that 100 barrels was added in Table 217, but the extract was increased by use of 24 cwt. of sugar in this case. It is advisable not to reduce the gravity of copper worts much below the figures given in Table 217, and to add the necessary quantity of liquor in the fermenting vessels to adjust the gravity at collection. Liquor added to the copper or fermenting vessels should be treated in the same way as mashing liquor or, at least, well boiled to remove excess of carbonates and allowed to clarify by settling before use.

The length of wort measured just before casting the copper is referred to as "copper out." This is measured at 212° F., so that it is necessary to reduce it by 4% to find the length at 60°, if it is desired to find the extract in the copper from the specific gravity of the wort, determined at 60° F. The allowance made for mash tun losses in the Table covers retention by the grains of 48 barrels of last runnings at 1004 sp. gr. It has been assumed that three-quarters of the wort absorbed by the spent hops is recovered with the hop sparge. This should not exceed about 6 barrels per cwt. of hops, any further reduction of the gravity of collected wort being made by addition of liquor.

TABLE 217.—EXTRACT LOSSES AND VOLUME CHANGES IN A BREW

50 quarters malt—Extract 97 lb. per quarter.
24 cwt. sugar—Extract 72 lb. per 2 cwt.
4 cwt. hops.

Losses and volume changes	Brls.	Yield		at 60° F.		Extract lb. per Qr. materials
		degrees	Brs. lb.	sp. gr.	lb.	
Mash, 6·76 brls. per quarter	338	13,470	4850			97·0
Grains, absorb 0·86 brls. Qr. malt	43	172	62			
Wort, brls. at 212° F.	295	13,298	4788	1047 0	16·9	95·7
Liquor added with 24 cwt. sugar	100	2,400	864			
Copper in, at 212° F.	395	15,698	5652	1041·4	14·9	91·1
Evaporation 15% in 1½ hr.	59	—	—			
Copper out, at 212° F.	336	15,698	5652	1048·7	17·5	91·1
Hops, absorb 2 brls. cwt. ..	8	374	135			
	328	15,324	5517	1048·7	17·5	89·0
Cooling, shrinkage 4% evaporation 5% loss in plant 1%	33	153	55			
	295	15,171	5462	1051·4	18·5	88·1
Hop sparge and liquor ..	45	280	101			
Collected in F.Vs.	340	15,451	5562	1045·5	16·3	89·7

(522) Making up the Lengths.

The foregoing is an example of the simplest case presented in making up the wort lengths. Though the volume shrinkage from copper to fermenting vessel may not be so great as that given in the Table, and the extract loss greater, the gravity of wort collected in the fermenting vessel should be higher than that found before casting the copper. It should be safe to make sure that the latter is not lower than the required final gravity. Two calculations may be necessary: (1) to find the evaporation required to bring the gravity of "copper in" wort to a suitable "copper out" gravity, and (2) the length of hop sparge and liquor required to adjust the gravity of collected wort in the fermenting vessels. The length to cast from the copper or evaporation required can be found by multiplying the volume collected in the copper by its gravity and dividing by the gravity required. Calculation of the length of hop sparge required is identical with that for determining the proportions in which two worts shall be mixed to yield a wort of intermediate gravity.

The formula is

$$\text{Barrels sparge} = \frac{\text{Barrels wort (wort gravity} - \text{required gravity)}}{\text{required gravity} - \text{sparge gravity}}$$

This becomes in this case

$$\text{Barrels sparge} = \frac{295\ (51{\cdot}4\ -\ 45{\cdot}5)}{45{\cdot}5\ -\ 6{\cdot}2} = 44{\cdot}3$$

395 barrels of wort would usually be boiled in more than one length. In these circumstances, the gravities of the worts in the different coppers should be equalised as nearly as possible for reasons given in Section 480. The sugar and hops can then be divided equally between the three coppers. An appropriate quantity of the strongest mash tun worts should be held back in an underback or copper pan for blending with the weakest worts. Enzymic action must be prevented by raising it rapidly to the boil. If three coppers are to be used for the wort of Table 193, the gravities could be equalised by holding back 40 barrels of the first mash tun worts for the third copper. The remainder of the wort would be collected in the first and second coppers, as it runs from the mash tun, 80 barrels of liquor being added to the first and 20 barrels to the second, according to the following approximate figures.

1st copper 58 barrels wort, 8 cwt. sugar, made up to 138 barrels = 5,677 degrees.
Copper out 117 barrels at 1050·7.
2nd copper 107 barrels wort, 8 cwt. sugar, made up to 127 barrels = 4,885 degrees.
Copper out 109 barrels at 1047·0.
3rd copper 40 barrels strong wort, 8 cwt. sugar with weak wort to 130 barrels = 5,136 degrees.
Copper out 110 barrels at 1048·4.

Total length cast from coppers is 336 barrels = 15,698 degrees. These are the same as given in Table 217, and the fermenting vessels could be made up to give a wort of 1045·5 by dividing the coppers equally between them and similarly dividing the 45 barrels of liquor and hop sparge.

(523) Parti-gyles.

Brews divided in the fermenting vessels to make worts of different gravities are known as parti-gyles. That described in the previous Section could, for example, be divided, among other ways, to produce:

100 barrels at 1050 = 5,000 degrees
100 barrels at 1040 = 4,000 ,,
200 barrels at 1032 = 6,400 ,,
15,400 ,,

The extra length can be obtained by adding more liquor to the coppers or by boiling the necessary length of treated liquor and passing it over the refrigerators to add to the wort in the fermenting vessels. It is possible to produce many parti-gyles from one copper length by adjusting the gravity of the weaker wort with liquor in the fermenting vessels. It is, however, more usual to collect more than one copper at different gravities, and blend them in the fermenting vessels. The second or third coppers must then be of considerably lower gravity than the first. If the worts were divided in the coppers as they ran from the mash tun, they would necessarily be of abnormal composition, with proteins, salts, etc., very unequally divided. As a result, the break in the last copper might be defective, the fermentation of the weaker worts liable to suffer and the flavour of the beer not so good as it should be. A boiling fermentation frequently occurs in a weak wort made in a parti-gyle with a strong one. It is consequently advisable to hold back some of the stronger mash tun worts for the later coppers, even though it may be inconvenient to equalise their gravities.

If the mash tun worts are run as shown in Table 193, the first copper would yield 9,484 degrees of extract and the second 3,814; 8 cwt. of sugar added to the first and 16 cwt. to the second, give 10,284 and 4,214 degrees, respectively. The "copper in" lengths, with 100 barrels of liquor in the first, would be 215 and 180, or about 206 and 173 barrels of cold worts, with gravities of about 1050 and 1024·4. The last gravity is rather low for the second copper. It would be better to hold back the first 20 barrels of mash tun wort for the second copper, when the "copper in" lengths would be 195 and 200 barrels. With 8 cwt. and 16 cwt. of sugar in the first and second coppers, the "copper in" gravities and yields would be, on cold worts,

187 barrels at 1045·7 = 8,546 degrees
192 barrels at 1037·25 = 7,152 ,,
15,698 ,,

The hops are divided between the coppers in proportion with the extracts, say 245 and 203 lb. The worts collected in the fermenting vessels might be expected to be as follows, with 25 barrels of hop sparge and 80 barrels of liquor, taken at a specific gravity of 1004,

146 barrels 1st copper wort at 1056 = 8,176 degrees
149 barrels 2nd copper wort at 1046 = 6,854 ,,
105 barrels hop sparge and liquor 1004 = 420 ,,
15,450 ,,

It is difficult to work out the proportions of the two worts and liquor to make the three lengths and gravities required, since the gravity of the first copper only is known when the proportions in which it must be divided have to be decided. A first approximation can be found by the following simple method.

The differences between the gravities of the first copper wort, second copper wort and sparge, and the required gravity, taken in reverse order, give the proportions in which the two worts and sparge must be mixed to give that gravity. Thus to obtain 1050 from a first wort of 1056, with second wort and sparge estimated at 1046 and 1004, the proportions are: 1st wort 46, 2nd wort 4, sparge 6. The respective lengths to make 100 barrels at 1050 would be 83·1, 7·1 and 10·8 barrels. This device cannot be used for the 1050, 1040 and 1032 worts simultaneously, since it takes no account of the available copper lengths.

The following figures are obtained by adopting it for the two stronger worts and assigning the estimated surplus copper and sparge lengths to the 1032 wort.

	1050		1040		1032	
1st copper	82 × 56 =	4,592	62 × 56 =	3,472	2 × 56 =	112
2nd copper	8 × 46 =	368	10 × 46 =	460	131 × 46 =	6,026
sparge and liquor	10 × 4 =	40	29 × 4 =	116	66 × 4 =	264
	100	5,000	101	4,048	200	6,402
	100 barrels at 1050		101 barrels at 1040·1		200 barrels at 1032·0	

It is obvious that the composition of the 1032 wort leaves much to be desired, on account of the excess of second copper wort. It is, indeed, essential to scrutinise all calculated blends in this way, since many of the possible variations may be unsatisfactory. A reasonably proportioned blend in one of the gravities may be accepted as fixed. The other two would be suitably adjusted. In this case, the 1050 wort is taken as the starting point.

The other two are adjusted by reducing the 1st copper length in the 1040 wort and increasing that in the 1032, with corresponding alterations in the sparge lengths to produce the required gravities, giving the following figures,

	1050		1040		1032	
1st copper	82 × 56 =	4,592	30 × 56 =	1,680	34 × 56 =	1,904
2nd copper	8 × 46 =	368	50 × 46 =	2,300	91 × 46 =	4,186
sparge and liquor	100 × 4 =	40	21 × 4 =	84	74 × 4 =	296
	100	5,000	101	4,064	199	6,386
	100 barrels at 1050·0		101 barrels at 1040·2		199 barrels at 1032·1	

(524) Calculation of Extract, Hop Rates and Costs.

Coppers and fermenting vessels are gauged in such a way that the volume of their contents can be measured by determining the "dry dip" by means of a "dip stick." The latter is a graduated rod, with a short metal arm at right angles to its length at the zero point. The projecting arm is laid flat on a small metal "dip plate" fixed on the side of the vessel. The dip stick is thus held vertically in the vessel. The highest graduation mark moistened by the wort indicates the dry dip, since the graduations commence at the projecting arm, placed on the dip plate. Dips are read to one-tenth of an inch. The corresponding volume of wort in a fermenting vessel is then ascertained by reference to a book prepared by the Excise Officer after he has accurately gauged the vessel. Coppers are not necessarily so accurately gauged.

The volume of each wort collected in a fermenting vessel from different coppers is ascertained in this way and the specific gravity determined by means of a saccharometer. Instructions are given to run the second and subsequent lengths to a definite dip, after the necessary calculations have been made from the previous dip and gravity. It is not a simple matter to obtain a truly representative sample from a fermenting vessel containing mixed worts, but a satisfactory running sample can be taken by skilful use of a can drawn through the worts by means of a string. The final dip and gravity of the collected worts are entered in the Excise book as the declaration for the purpose of duty charge.

It is usual on completion of a brew to calculate the yield of extract, the hop rate per barrel and the cost per barrel of beer.

The extract may be that for the whole of the materials, as in Table 217, but that obtained from the malt is generally estimated, after deduction from the total yield of a suitable allowance for adjuncts and, in some cases, for coloured malts. This allowance may be 277·8 degrees or 100 lb. per quarter of flakes, and 200 degrees or 72 lb. per 2 cwt. of invert sugar, for example. The extract of the materials is given by

$$\frac{\text{Barrels collected} \times (\text{specific gravity} - 1{,}000)\ (\text{or brewers' lb.})}{\text{quarters of materials}}$$

That for the parti-gyle in the previous Section would be

$$\frac{5{,}000 + 4{,}064 + 6{,}386}{62} = 249{\cdot}2 \text{ degrees or } 89{\cdot}7 \text{ lb.}$$

The extract of the malt would be regarded as

$$\frac{15{,}450 - 2{,}400}{50} = 261 \text{ degrees or } 94{\cdot}0 \text{ lb.}$$

The latter is compared with the laboratory extract of the malt.

The hops were divided in such a way that each barrel of first copper wort would contain the extract from 1·68 lb. and each barrel of second copper wort that from 1·36 lb. The hop rates of the three worts thus work out at 1·39, 1·17 and 0·91 lb. per barrel, respectively.

The cost of the hops and sugar must be similarly apportioned to the three beers of the parti-gyle. That of the malt may be divided in accordance with the extracts collected, after making allowance for the proportion derived from sugar, which would differ in the three qualities. The duty, calculated from the length and gravity of each wort, is added after making the statutory allowance of 6% for loss. The actual loss at racking should not amount to more than 2 or 3%, provided the casks are properly gauged and excessive over-measure avoided. The cost of the beer per barrel is finally given as the sum of the materials cost and duty charge. The actual loss from fermenting vessel to cask should be considerably less than the statutory 6% allowance. It is generally 3 or 4%, with casks holding ~~two~~ quarts extra per barrel. It reaches 6% with bottled beer.

(525) Extract Yield Per Cent.

By yield, in breweries using the Plato or Balling tables, is understood the weight of dry extract in the wort, expressed as a percentage of the weight of materials used. The saccharometer gives the extract in wort as a percentage of its weight, but wort is measured, not weighed. Hence it is necessary to convert Plato or Balling % to volume %, that is grams extract per 100 ml. wort, in order to find the weight of extract in a given volume. The volume percentages are given in some tables, but can always be found by multiplying the Plato or Balling % by the corresponding specific gravity. Thus 12·612% Plato, corresponding with 1·0510 sp. gr. at 17·5°/17·5° C., is equivalent to 12·612 × 1·051 = 13·255 volume %.

The brewhouse yield is frequently calculated from the saccharometer reading of a sample taken from the copper just before turning out, and cooled to 17·5° or 20° C., according to which temperature is used for the Plato or Balling Table employed (Vol. I, Section 242). A correction of 4·2% for contraction between 100° and 17·5° C., or of 3·83% between 95° and 17·5° is made, with 0·3% addition for expansion of the copper, and a deduction of 0·8 litre for volume displacement by each kgm. of hops. The round figure of 4% is used to cover all these. The wort length at 17·5° C. may thus be assessed by multiplying the volume of boiling wort in the copper, just before turning out, by 0·96. The materials yield, or length required when casting the copper, can be calculated by substituting the known values in the equation,

$$\text{Kgm. materials} \times \text{extract materials} = \text{litres boiling wort} \times 0{\cdot}96 \times \text{volume extract}$$

$$\text{e.g., } 5{,}000 \times 72 = 28{,}302 \times 0{\cdot}96 \times 13{\cdot}25.$$

This would mean that the brewhouse yield of the materials, giving 283 hl. wort when casting the copper, with an extract of 12·6% Plato (= 13·25 volume %), was 72%. Alternatively wort from 5,000 kgm. malt, with an extract of 72%, would have to be boiled down to 283 hl. to give wort with a saccharometer reading of 12·6% Plato, at 17·5°/17·5° C. The extract obtained is usually 1 to 2% lower than that determined by the Congress analysis.

An approximate formula, by Wahl, for use when the materials are weighed in pounds and the wort measured in American beer barrels, for the Balling saccharometer, is

$$\frac{(259 + B) \times B \times \text{bbls.}}{\text{lb. materials}} = \text{yield } \%$$

This is derived from the weight of an American beer barrel of water at 5° C., which is 258·5 lb., and the fact that a barrel of wort at B Balling weighs approximately B lb. more than this.

The volume contraction and extract losses already given are those generally accepted in lager breweries. The length collected in the fermenting vessel averages about 10% less than that cast from the copper, while the extract may be about 1% greater than that of the copper wort. A further loss of about 5% occurs during fermentation and storage, so that the volume of beer racked may be 15%, or rather more or less, than that of the wort turned out of the copper. The " copper in " length would be calculated from the average evaporation rate.

BRITISH EXCISE REGULATIONS

(526) Licences.

There is a licence for Brewers for Sale and a duty on the beer brewed. The former costs £1 in the first place, but the charge on renewal on October 1st each year is £1 for the first 100 barrels and 8/- for every further 50 barrels or part thereof. These figures are based on bulk barrels, less 6% for waste. The term bulk barrel refers to the volume of wort collected in the fermenting vessel, regardless of gravity. " Standard barrel " signifies the equivalent number of barrels at a specific gravity of 1055. This term originated when duty was charged on the basis of wort collected at 1055 specific gravity. A brewer who deals in beer from another brewery, whether his own or another brewer's, must also hold a Wholesale Licence, costing £10 a year. The regulations and charges given were those in force in September, 1939.

(527) Entries and Declarations.

Premises and Vessels.—The premises of the Brewer are surveyed by the Excise authorities. Every mash tun, and every collecting vessel, whether it be fermenting vessel or sugar solution vessel, is numbered and gauged. Every copper, wort receiver, hop back, cooler, refrigerator, cleansing back, yeast back, racking tank, tun room and beer store is numbered. Every place where malt, corn or sugar is kept is marked.

Materials.—Under the Beer Act of 1880 no material may be used in brewing other than malt, grain, sugar and hops unless the sanction of the Commissioners of Excise is obtained in writing. If they do grant permission to use any other substance they may consider it as sugar.

Notice to Brew.—A White Book is supplied by the Excise authorities in which the brewer must give 24 hours' notice of his intention to brew or dissolve, and at least 2 hours' notice of the amount of malt, corn, sugar and hops it is intended to use. The time when all the wort will be drawn from the grains must also be stated, and the grains must not be cleared from the mash tun until 1 hour after this time.

The White Book must be available for inspection at any time, in order to allow the Excise Officer to be present, if he wishes, at any dissolving or mashing operation and to check the quantities of materials being used.

Declaration of Worts.—The brewer must enter in the same book within one hour of collection of every brew and sugar dissolving the dip of the vessels and the true original gravity of the wort or sugar solution. If collection is made after 9 p.m., the entry may, however, be delayed until any time before 9 a.m. the next day. The specific gravity is entered to one degree as determined by the saccharometer. Fractions of a degree are ignored. The period occupied in collection of worts may not exceed 12 hours. Primings and caramel must be dissolved, collected and declared in the manner prescribed for worts. The dip and gravities are generally checked by an Excise Officer who may then state that the vessel is " cleared," in other words, its contents may then be moved. In the absence of an officer, worts and sugar solutions may not be moved for 12 hours. The gravity is taken by the Excise officer by means of a saccharometer. He is entitled to surcharge the brewing by raising the declared gravity, if he finds that the original entry was incorrect. He is the sole judge in this matter and is entitled at any time to take samples for determination of original gravity at the Government Laboratory.

No wort or priming solution may be collected at a higher gravity than 1150.

(528) Duties.

Beer Duty.—The basic rate of duty from 1935 to September, 1939 was 24/- per barrel of 36 gallons of wort of 1027 degrees of gravity, or under. The rate increased by 2/- per degree of gravity up to 170/- for a wort of 1150 degrees. An additional 24/- per barrel was levied in the emergency budget of September 27th, 1939.

An account of the quantity of wort collected at each gravity is kept by the officer in a private Blue Book and, at the end of the month, the total bulk gallons, less an allowance of 6% for waste, is assessed at each gravity. The beer duty account is made up on this basis, and is usually rendered on the 2nd of the month. Duty must be paid by the 15th.

A beer tax of 6/3 per "standard barrel" of 1057 degrees gravity took the place of the Malt Tax in 1880. The standard barrel was altered to 1055 in 1889. In 1894, the duty was raised to 6/9 and in 1900 to 7/9, at which it remained until 1914, when there was an increase to 23/– per standard barrel at 1055. In 1916, this was increased to 24/– and in 1917 to 25/–. In 1918 the duty was doubled and was raised to 70/– in 1919. From 1922 to 1930 it was £5 per standard barrel, but a rebate of 20/– per bulk barrel was allowed by the Budget of 1923, on beers of 1025 and upwards, with diminishing rebates between 1024 and 1014 degrees. Increases of 3/– and 31/– per standard barrel were made in 1930 and 1931. The duty then stood at £6 14s. per standard barrel, less 20/– per bulk barrel at 1019 and above, with lower rebates between 1018 and 1010 degrees. In 1933 the basis of duty was altered to the bulk barrel by abolition of the "standard barrel" and the duty was fixed as stated above.

Materials Charge.—42 lb. (1 bushel) of malt or 28 lb. of sugar are estimated to produce 18 gallons of wort or solution at 1055 sp. gr. The total yield of materials used is calculated by the Excise Officer at the end of each month and, should it fall short of the estimated produce, a charge based on the weight of materials used is substituted for that calculated from the declarations.

The Excise Officer counts the bags or casks of sugar and gauges the grains left in the mash tun, in order to keep a check.

Customs Duty.—The duty on imported beers from within the Empire is 25/3 per bulk barrel at 1027 sp. gr. and 45/3 on beer from foreign countries, with an increase of 2/– per barrel for each degree of gravity above 1027 in both cases.

Yellow Book.—The Excise officer may take the dip and gravity of worts in fermenting vessels at any time. He records these in a Yellow Book, of which the brewer is entitled to a copy. The officer may call for an explanation of any irregularities found.

Worts under 1027 *Gravity.*—Duty is paid at the minimum rate on worts intended for beer of between 1016 and 1027 gravity. A weak beer of less than 1016 original gravity, and with a proof spirit content of less than 2% may be brewed on not-entered premises. It is not liable to duty, but must not be described as beer, ale or stout.

(529) Blending in Fermenting Vessels.

Primings, Caramel and Worts.—If a brewer wishes to add primings or caramel solution to a fermenting vessel, or to mix two fermenting worts he must give notice in the White Book, with details of the vessels concerned. After mixing, he must give the dip and gravity of the mixture in the fermenting vessels. No notice is required for transfer to cleansing vessels or racking backs. Yeast may be added at any time. The addition of water to beer is prohibited.

Yeast Pressings.—Yeast pressings or barm ale may be returned or added to fermenting vessels in amount not exceeding 3% of their contents. A statement of the addition must be entered in the White Book.

Blending Worts under 1027.—If the Officer is informed of the intention to blend worts of specific gravity under 1027 with stronger worts, and the gravity of the mixture is above 1027, the charge may be adjusted to be on the bulk and original gravity of the mixture. If the Officer is not previously notified, the charge on the weaker wort will be that on the minimum of 1027. The mixture must be done from gauged vessels.

(530) Indulgences.

Permitted Materials.—The Excise may, if requested, give indulgences or permission to use in appropriate circumstances certain materials, other than malt, sugar and hops. Each officer has a list of materials which he may permit without further enquiry. These include : (*a*) malt extract and special sugars. These must be entered with the materials in the same column as sugar, but on another line. Their nature must be stated. They are considered to yield wort in the materials charge. (*b*) Yeast foods. The amount and time of addition must be entered in the White Book, but no account of them is taken in calculating the materials charge. They must be added to the brew before collection. (*c*) Finings may be added at any time, but it is advisable to submit all special and patent preparations of this nature to the Officer before use. (*d*) No preservative other than sulphurous compounds or sulphites may be added. The quantity added must be strictly controlled on the basis of their SO_2 content. The permissible limit is 5 grains per gallon or 70 parts per million of SO_2.

Prohibitions.—It is not permitted to add saccharine or liquorice to beer, as they yield a sweetness or fulness, which would possibly replace gravity and be a source of loss to the revenue.

Dry Hops or Hop Oil.—The amounts added must be entered in the White Book at least once a month. The hop oil must be approved before its use is permitted.

(531) Spoilt or Lost Beer.

Destroyed Beer.—Beer that has left the brewery premises and has become unfit for sale may be returned for destruction and repayment of duty, provided the appropriate declaration is made. Figures must be provided by the brewer showing all worts concerned in each brew affected, and particulars by which they can be identified, dates of brewing, dispatch and return, original gravities, quantities in each individual cask, with distinguishing marks, number of bottles, etc. The Excise will not permit less than 3 gallons per barrel to be included. The contents of all the casks are emptied into a gauged vessel and, after mixing, the dip and samples are taken before the beer is run to the sewer. A "Returns Book" of prescribed form must be kept, and full particulars entered on the day of receipt of all beer for which an application for return of duty will subsequently be made.

Accidental Loss.—If loss occurs through open cocks or similar accident, and the return of duty is to be claimed, the Excise Officer must be shown all the available evidence of loss, e.g. no froth, sediment or beer on the floor must be washed away until after his inspection.

(532) Sugar Stock.

The brewer must keep a "sugar stock book." He must enter on receipt of any sugar, (1) the invoice date and number; (2) the name and address of the supplier; (3) date of receipt; (4) the marks on each package, number of packages and weight in each; (5) the total weight of consignment. When the sugar is used, he must enter (1) the number of packages, weight in each and total weight disposed of; (2) the date and purpose of disposal, with particulars identifying the relative brewing or dissolving in which used.

The initial entry in the book must be agreed with the Surveyor of Customs and Excise. Thereafter the book must be balanced to show the number of packages and weight of sugar of each description, which ought to be in the sugar store at the close of each calendar month or any other time required by an officer of Customs and Excise.

The brewer may have no sugar in his possession elsewhere than in the sugar store or mash tun or other entered dissolving vessel, or in due course of removal thereto. He is not permitted to remove sugar from the sugar store, without prior consent of the Commissioners, except for the purpose of brewing or dissolving and in accordance with an entry in the appropriate book.

(533) Original Gravity.

The distillation process of analysis is officially accepted by the British Excise authorities for determination of the original gravity of partly fermented worts or beers, that is for ascertaining the specific gravity of the wort as collected in the fermenting vessel, before fermentation had commenced. The method is given in a later Section. The original gravity table, from which the specific gravity removed by fermentation is calculated from that of the spirit distilled from the beer and of the residue left after distillation, both having been made up to the volume of the beer taken for analysis, was empirically constructed by averaging the analyses of samples of partly fermented worts and finished beers from 34 brews in 10 breweries, carried out under the supervision of Sir Edward Thorpe and Dr. H. T. Brown[1] in 1909–1910. The samples were selected as representative of the different fermentation systems and materials used in British breweries. Variations in the inevitable slight loss of alcohol during fermentation, and other disturbing factors, render it impossible to find one single factor for the conversion of " spirit indication " to degrees of gravity lost, which shall be applicable in all cases. " Spirit indication " is the term given to the difference between the specific gravity of water, taken as 1000, and that of the spirit distilled from the beer and made up to the original volume of the beer. The errors involved in the application of the table constructed from the average of all the analyses, known as the " Mean Brewery Table," to finished beers of any particular brewery are very small, and do not exceed 0·5 of a degree of specific gravity, water being 1000·0. The results are usually slightly lower than the true original gravity, but rather higher in the case of lager beers. The low results arise from inclusion of some partly fermented worts among the samples from which the Table was constructed. The original gravities of worts, when calculated from the Table, are lower than the true original gravities by amounts varying from 0·15, when the spirit indication is 1·0, to 0·66 when it is 5·0 (Hopkins[2]). This is due to abstraction by the yeast of water and solids from the wort, and non-return of alcohol to the wort. (Section 595.) The slightly high results obtained for the original gravity of lager beers by the use of the official Table are due largely to two factors. Slight reduction of the loss of alcohol at the low fermentation temperature, and greater return of alcohol from the yeast to the wort, on account of the time that it remains in contact with the wort, than is allowed for in the Table, which was constructed primarily for top fermentation beers.

Among the disturbing factors that operate during fermentation and complicate the relation between the spirit indication and degrees of gravity lost, are changes in the solution divisor of wort constituents (Section 241), and increase in the specific gravity of the wort by inversion of cane sugar at the commencement of fermentation. Cane sugar exists in all malt worts, in quantities ranging from 0·5 to 1·0 grams per 100 ml. at specific gravities of 1045–1075. Each gram inverted increases the specific gravity of the wort by 1000·23, but this effect is eliminated by the time the alcohol content reaches 1·0 or 1·5%.

The decrease of gravity due to removal of solids by yeast is greatest in the early stages of fermentation. It is approximately represented by 4·5 times the dry yeast increase in 100 ml. It is useful to note that an approximation to yeast increase can be obtained by counting the cells in the unit volume, 1 /4,000 cubic millimetre, of a haemocytometer. One gram of brewery yeast, washed and pressed between folds of filter paper and then thoroughly mixed with water and made up to 100 ml., gives a count of 10 cells.[3] One cell per unit volume corresponds with 0·1 gram of pressed yeast or 0·022 gram of dry yeast per 100 ml. Laboratory fermentations of malt worts, with 2 cells per unit volume (0·2 gram pressed yeast per 100 ml.), showed that the loss of gravity due to a 9-fold reproduction was about 1·9 degree. In brewery fermentations, in which the pitching rate varied from 0·13 to 0·44 gram pressed yeast per 100 ml., and in which the yeast increase also varied considerably, the degrees of gravity lost ranged from 2·0 to 1·35, with an average of 1·6. It may be noted that there is a tendency in all fermentations for the yeast to increase to about 20 cells per unit volume of the haemocytometer. Hence the cell increase is less at greater pitching rates.

There may be loss of alcohol and water by evaporation during storage, and slight changes of gravity due to absorption by the wood, or transfer of water from the wood. Production of volatile by-products and acid may also lead to small differences between the ascertained and true original gravities. The empirical manner in which the Table was constructed allows for the normal acidity of new beer, but should this rise above 0·1 %, calculated as acetic acid, it is necessary to add to the spirit indication a quantity equivalent to the increase of specific gravity of the distillate caused by oxidation of alcohol to acetic acid. These corrections are given in a special Table.

The specific gravity of mixtures of alcohol and water does not diminish *pari passu* with increasing concentrations of alcohol. Equal increments of spirit indication, therefore, do not correspond with equal increments of alcohol in the distillate. Alcohol percentages must be calculated from Tables giving the specific gravity of the distillate with the corresponding percentages by weight or volume of alcohol, or percentages of proof spirit. There is, however, a nearly linear relation between the "degrees of gravity lost," that is the fermented specific gravity equivalent to the spirit indication, or approximately the difference between the original and residue gravities, and the alcohol content, which is given by grams alcohol per 100 ml. × 8 = degrees of gravity lost. This holds for alcohol contents up to 4·0%. Between 4·0 and 5·5%, the factor becomes 7·9, and between 5·5 and 7·5% it is 7·85.

An approximate figure for the alcohol content of beer, per cent. by weight, can be obtained by multiplying the difference between the original and present gravities by 0·102.

The original gravity may also be determined from the refractive index of beers (Section 243) with sufficient accuracy for many purposes, and much more rapidly than by the distillation process. It is also possible to derive the original gravity with fair accuracy from the present gravity of the beer and its boiling point, since the latter depends mainly on the alcohol content, the influence of the dissolved extract in beer being very slight. The boiling point of liquids varies with the barometric pressure. Allowance is made for this in Long's Alcoholmeter, which consists of a small boiler surmounted by a reflux condenser and thermometer, by means of a sliding scale on the thermometer. The scale is adjusted so that its zero point corresponds with the top of the mercury column, when water is boiled in the instrument under prevailing barometric conditions. A measured quantity of beer is then placed in the boiler, together with a small piece of soap to prevent frothing, and heated with a spirit lamp. The highest point to which the mercury rises in the thermometer is read on the scale, which is graduated to read degrees of gravity lost during fermentation. The reading added to the present gravity of the sample gives the original gravity.

The original extract of the wort from which a beer was obtained can be calculated from the extract of a beer and its alcohol content. This method is used in lager breweries where the Balling or Plato Tables are used, the extract and alcohol being expressed as grams per 100 grams. The calculations are based on relations established by Balling, according to which 2·0665 grams extract yield 1 gram alcohol, 0·9565 gram CO_2 and 0·11 gram yeast. For each gram of alcohol formed 1·0665 gram of CO_2 and yeast is added to the beer, so that the original extract of the wort, in grams per 100 grams or % Plato is given by :

$$p = \frac{(A \times 2{\cdot}0665n) \times 100}{100 + A \times 1{\cdot}0665}$$

in which p = extract of the wort, n = extract of the beer, and A = alcohol, all as % by weight (grams per 100 grams).

The following approximate relations serve to calculate the real attenuation and alcohol content of beer, when the original extract of the wort and the extract of the beer are known in % Plato.

Alcohol % by weight = 0·42 × apparent attenuation (extract wort − extract beer)
Real attenuation = 0·84 × apparent attenuation.

(534) Summary.

Accurate calculation of the materials required for a brew and division of the copper worts according to the gravities of the beers required is very important. It is desirable to equalise the gravities of different copper lengths, in order to obtain consistent protein and mineral wort composition. When it is necessary to make up strong and weak coppers for parti-gyles, care must be exercised to ensure satisfactory composition of the weaker worts collected in the fermenting vessels, as well as to obtain the necessary lengths and gravities. If the yield obtained from malt agrees badly with the analytical determination of extract, the reason for the loss of extract must be sought. It may be found in defective milling or mashing and should not exceed 1 or 2%. Since the original gravity of beers is determined by the use of tables constructed empirically from determinations made at a number of breweries, it may happen that the results will not accurately agree with the known original gravity in a particular brewery. Consistent differences may be due to a lower loss of alcohol during fermentation than the average allowed for in the tables. This may happen in fermentations in closed vessels or with lager beer, fermented at a low temperature. In such circumstances the analytically found original gravity would be slightly higher than the true original gravity.

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1. E. Thorpe and H. T. Brown, *Journ. Inst. Brew.*, 1914, **20**, 569.
2. R. H. Hopkins, *Journ. Inst. Brew.*, 1927, **33**, 320.
3. A. Tait and L. Fletcher, *Journ. Inst. Brew.*, 1923, **29**, 509.

FERMENTATION

CHAPTER XXXI

FERMENTING ROOMS, STORAGE CELLARS AND THEIR EQUIPMENT

(535) Fermenting Rooms and Cellars.

A germ-free atmosphere, at constant temperature and humidity, with means for renewal sufficiently frequently to keep its CO_2 content below 3% is an essential feature of modern fermenting rooms and cellars. Air-conditioning from a large central station or small units is advisable in all cases and preferable to coil refrigeration for lager fermenting cellars. The room or cellar must be closed to prevent inflow of dust-laden air from other departments and hot or cold air from outside, but the slight overpressure of conditioned air should be sufficient to permit of the use of ordinary, well-fitting doors and windows. The latter should be double to prevent condensation in cold weather with top fermentation temperatures. Floors must be made of hard wearing, readily washable material. The walls, rounded at the floor and ceiling, should also be finished in washable material. There should be no beams or mains from which a drip into fermenting vessels is possible or on which dust may collect. The photograph in Fig. 136 represents a bottom fermentation cellar, with concrete vessels of the Rostock system. The entrance for refrigerated and filtered air is near the ceiling and the outlets on the floor at the sides of the fermenting vessels. Fig. 137 is a photograph of a top fermentation room, in which the fermenting vessels are enclosed in a glass-fronted chamber, through which purified air is passed. Fig. 138 is an air-conditioned fermenting room, with Dukeron enamelled copper vessels. Yeast is removed by suction through pipes passing through the front of the vessels.

FERMENTING VESSELS

(536) The Material of Fermenting Vessels.

It is impossible to specify any one material as the most suitable for fermenting vessels under all conditions. The ideal material must not harbour micro-organisms or preclude reasonable methods of sterilisation. Biological contamination of wort can only be avoided by impermeability, absence of cracks or crevices and a surface from which hardened deposits of yeast or beerstone can be removed readily and without damage. Essential chemical properties include resistance to corrosion and attack by fermenting wort or beer, with possible detrimental effects on yeast growth, even when the attack is too slight to cause any harm to the flavour or hygienic properties of the beer. The material must also be capable of withstanding suitably selected cleansing and sterilising agents. Heat transmission through the sides and bottom of the vessel should be low, and it is necessary to avoid electric currents or couples by the juxtaposition of different metals or impurities in metal sheets.

Against these desiderata must be set the question of cost, ease of construction, strength, durability, alterations, repairs and applicability to the system of fermentation employed or particular circumstances. Cost and convenience may depend on the possibility of erecting the vessels *in situ.* Considerable risk is involved in the transport of some types of completed vessels and others may be too large to take into existing rooms or cellars, without extensive demolition of walls, etc. The possibility of carrying out repairs and alterations in size or position, with available facilities, should not be overlooked, while availability in the district becomes important with such materials as slate.

No one material would appear to comply with all these requirements but, in many cases, an admirable combination of desirable properties can be secured by making use of suitable linings. Certain materials have proved perfectly satisfactory in some breweries, but defective in others, for reasons which cannot always be readily traced, though, in many cases, the failures are due to lack of experience in construction or erection, or to insufficient purity or homogeneity in the material. Experience in use is also a factor in selection from the wide range of possible materials, while the size of the vessels may rule out some or make others appear more suitable. Modern plastic materials do not yet appear to have been used for fermenting vessels.

The design of vessels for various fermenting systems, and the devices adopted for removing the yeast after fermentation, are described in Chapter XXXIII. The depth of wort is, in some circumstances, of considerable importance. Settlement of non-flocculating yeasts or clarification, after the rising yeast has been skimmed, is considerably delayed in very deep vessels. The wort depth is consequently restricted to about 4 feet 6 ins. or

5 feet, when yeast of this type is employed, but may be greater with yeasts which settle more rapidly.

(537) Heat Transmission.

Comparative figures for heat transmission by a few materials used in the construction of fermenting vessels are given in Table 218. They reveal the very great difference between wood and concrete, on the one hand, and metals, on the other. A low heat transmission may be very advantageous when it is necessary to retain the heat of fermenting wort, though it offers resistance to external cooling. A rather higher room temperature is maintained in lager fermenting cellars equipped with metal vessels, than in corresponding cellars with wooden vessels, to counterbalance the greater heat loss from the former.

TABLE 218.—HEAT TRANSMISSION OF VARIOUS MATERIALS

Material	Thickness mm.	Coefficient of conductivity	Loss of cold Kg. cal/hour/m^2/°C
Oak	50	0·17	3·4
Concrete F.Vs. ..	150	0·65	4·3
„ tanks ..	250	0·65	2·6
Steel	10	56·0	5,600
Aluminium ..	6	175·0	29,200

(538) Surface of the Material.

A smooth polished surface gives the greatest security from infection, while immediate removal of scale after use, and surface polishing, helps greatly in the prevention of corrosion with such metals as aluminium. Experience has, however, shown that a very smooth surface is not altogether an advantage in fermentation. An increased tendency to yeast sedimentation, diminished outcrops and defective clarification have been noticed in top fermentation breweries, where a change has been made from wood to polished metal vessels. The effect is to be attributed, partly, to restricted evolution of carbon dioxide from smooth surfaces, in comparison with the readiness with which the bubbles are formed and disengaged from rougher materials. It has also been noticed in bottom fermentation breweries that fermentation starts more quickly when the sides of the vessel are covered with beerstone, largely calcium oxalate, than when the surfaces are very smooth. Generally the attenuation is less with easily wettable, smooth surfaces, such as enamel, than with wood or wax which resist wetting. Materials have been placed by Emslander [1] in the following order of decreasing rate of fermentation and attenuation; wood, shellac, varnish, paraffin wax, aluminium, pitch, glass-enamel, glass. Some lager brewers find that two days longer are required for fermentations in glass-lined steel vessels than in Mammut-lined metal vessels. Settlement and clarification is generally more rapid in wooden storage vessels than in glass-lined or metal tanks, though this is influenced in most cases by the greater size of the latter.

(539) Electrical Currents in Fermenting Vessels.

Some of the surface effects mentioned in the last Section are attributed to electrical actions. A negatively charged wax surface will attract positively charged carbon dioxide and colloid particles from the wort. These are discharged at the negative surface, to give neutral gas bubbles and precipitated colloids. Enamel surfaces are, on the other hand, positively charged and repel these substances. More rapid appearance of kräusen at the surface of the wort above copper attemperators than elsewhere in enamelled iron vessels is sometimes noted. The very small currents which may normally pass through fermenting worts in metal vessels are, however, not likely to have any injurious effects,[2] although greater currents have an influence on the stability of colloids and on the yeast. The most serious effects of electrical currents are the corrosions which may result when they are set up between two metals, copper attemperators and aluminium vessels, for example. Great care is taken in modern constructions to avoid all such risk of damage.

(540) Wood.

Until comparatively recently, wood was almost universally used for fermenting vessels, slate and stone being its only serious competitors, though iron was used to a restricted extent before the general introduction of other metals in the latter half of the nineteenth century. Possibly no material so well satisfies the majority of the requirements laid down as hard, closely grained, well seasoned wood, selected for its freedom from faults. It is, however, always more or less porous and, in use, provides gradually increasing facilities for absorption of wort, with inevitable risks of infection. Despite this, many brewers still maintain that no material is so good for fermenting vessels, when carefully maintained. Fermentations are regular and satisfactory, while heat transference is small. No toxic effects on yeast are to be feared and the flavour of beer is unaffected, providing the wood is properly selected and well seasoned. Cheapness in relation to first cost and durability, convenience of erection and repair have all conduced to the favour in which wood is still held in many quarters, while the danger of infection has been minimised by the use of such linings as pitch, varnish, enamels and wax. Metal-lined wooden vessels are largely used, but may be considered as a makeshift for metal construction. Metal lining of old vessels is to be deprecated, as decay of the perished wood behind the metal rapidly increases. The most suitable woods are oak, kauri pine, red deal and cypress.

Both oak and fir have been largely used in Britain, but they are coarse in grain, contain many knots and become roughened with constant cleaning. New Zealand kauri pine has proved to be the most suitable wood, on account of its hardness, close grain and freedom from knots. Care must be taken to avoid swamp kauri which has been buried, for centuries perhaps, and is absorbent. The timber must, in any case, be weather-seasoned and free from sap. Non-corroding metals should be used for internal bolts and rods, particularly with kauri pine.

Round vessels are easier to clean and have a longer life than squares, but take up more space. Kauri pine and oak can safely be used without lining, but softer woods are generally varnished, enamelled or waxed. New vessels should be seasoned with cold water, containing a little salt. Hot water and cleansing agents should not be used for cleaning, since they tend to soften the wood. It is better to soak the sides with cold liquor, immediately they are emptied, scrub with fine pumice powder and spray with some sterilising agent, such as formalin, and allow to stand before the final wash. The upper part of the sides is usually coated with a paste of bisulphite, to prevent adherence of yeast. The whole surface should be planed, when patches of scale have formed, and it is a good plan to " dress clean " the wood with a blow-lamp if it becomes badly scaled and softened. It is advisable to leave wooden vessels out of use for a few days occasionally, but liquor should not be left on the bottom, as this softens the wood. Under normal conditions, wooden vessels may remain serviceable for 20 or 25 years, but the pores gradually become more open and offer shelter to micro-organisms. Infection from this source is extremely difficult to combat, even by means of blow-lamps.

Oak storage casks have given immense service in Continental lager breweries, but their size was restricted. Pressure vessels, made from 3- or 4-inch planks of redwood, cypress or oak, are still largely used in America, some of great capacity. Redwood is satisfactory for pressures up to 7 lb. per square inch. Cypress will withstand 10 lb., but oak is preferred for pressures up to 15 lb. The heads are braced with timber to prevent bulging and connected by brace rods, which are most frequently made of black iron, varnished with the tank. Upright tanks are carried by wood blocks on cast iron stands. The wooden saddles, formerly used for horizontal storage casks, are now generally replaced by galvanised bands or saddles, supported on stands. Closed tanks are fitted with cast iron manhole doors, varnished with the tanks.

The surfaces of oak and kauri pine fermenting vessels are commonly exposed to the beer in English breweries, but the softer woods are generally coated in some way to prevent absorption of wort or beer into their pores. As the vessels become older and the wood softens, it is impossible to sterilise them by means of hot liquor or antiseptics. Deterioration of the wood is prevented to a large extent and a sterile surface secured by coatings of spirit varnish or enamels, which must be applied when the wood is perfectly dry. The varnish is made by dissolving shellac in alcohol. The wood is covered with two coats, when hot, and the varnish should be renewed yearly, after mechanical removal of the old coat. The enamels are prepared from such materials as pitch or bitumen, dissolved in suitable solvents, of which trichlorethylene is an example. These are sprayed or painted on and burnt into the wood. Brewers' pitch is made from the crude resin of coniferous trees, which is melted and heated to drive off the contained water and oil. Crawford's, Dukeron & Mammut and Tetley's are examples of proprietary enamels ; various mixtures of pitch and wax are used. They are insoluble, give no flavour to beer and are reasonably durable, but must be replaced as the surface becomes worn, generally every six months. Coatings of paraffin wax, melting between 120° and 130° F., are used by many brewers. It sinks more deeply into the wood than varnish, particularly when the wood is well warmed. It forms a durable surface, which can be washed with hot liquor, but it is rather soft and bottom yeast does not settle on it as easily as on varnish. The wax is heated to between 150° and 190° F. and is poured from a ladle over the sides and bottom of the vessel.

(541) Slate.

Few fermenting vessels, built of the smooth, sandstone slabs, several inches in thickness, that gave their name to the Yorkshire Stone Square system, still remain in use. Their place has been taken by vessels built of slate or, more recently, of aluminium. It has, thereby, been made possible to increase the size of the vessels, while maintaining the methods of the original system, in their entirety or in part. Slate can be obtained in larger slabs than the stone, 1½ to 2 inches in thickness, so that a great deal of weight is saved. It has many of the advantages of wood, without its great disadvantage of porosity. It is very durable but, in time, tends to crack and flake, giving places which are difficult to keep clean and free from infecting organisms. A mixture of white and red lead with linseed oil or gold size is used for filling joints and cracks or around the manhole collar, which was formerly fixed in place with Portland cement. Slate is used in both top and bottom fermentation breweries, but is generally restricted to localities favoured by local supplies or sufficiently near the quarries to justify the cost and difficulty of transport. Slate vessels are necessarily rectangular, but jointing can be done so efficiently and pointed so smoothly with a paste of litharge and glycerine, that very large vessels can be satisfactorily erected.

A special design was developed in the Yorkshire system, Fig. 163, and was typically restricted in capacity by the size of the available slabs to between 30 and 60 barrels. Elsewhere slate is used for the more ordinary open squares. Its low coefficient of heat transmission and cleanliness make it very suitable for yeast backs, for which it has been very largely used in conjunction with wood or metal fermenting vessels in top fermentation breweries.

Beerstone forms rather rapidly on slate vessels, so that the cleaning methods are directed largely to keeping it in check, while great attention must be given to corners, joints and roughened places. The vessels are cleaned out immediately after use and brushed over with a thick wash made from quick-lime. This softens newly formed scale, which is then rubbed off with a pad of hop sack and gritty sand in conjunction with a jet of cold water. A slab of sandstone is also used with the sand, if necessary. When scale has accumulated it may be necessary to paint with a strong lye of one part caustic soda and two of quick-lime, which is allowed to act for 24 hours and is followed by scrubbing and scraping. The bottom of the vessels should finally be dried with a cloth before filling. Covered vessels should be filled with SO_2, if left empty for any time.

(542) Concrete.

Reinforced concrete is largely used in the construction of fermenting vessels and storage tanks. Its weight necessarily militates against its use in buildings originally constructed to carry vessels of lighter materials, but its strength is invaluable for vessels in new breweries in which they can form an integral part of the structure. The permanent nature of concrete vessels may prove a disadvantage if alterations should be required, and difficulties were at first experienced in surfacing or lining their interiors, pitch and paraffin wax not being suitable. These difficulties have been overcome in the Rostock and Borsari systems, with their Australite and Ebon linings, which provide a perfectly smooth, black surface, resistant to beer or even acids and alkalis. These linings have good wearing properties and are easily repaired. The concrete is impregnated to a depth of 5 or 6 mm. with the lining material of the Rostock system, while the Ebon lining is made in plates, 10–12 mm. thick. The concrete is prepared for the latter by covering with wire netting, on which a layer of resin or asphalt is laid. The plates are surfaced at the back with sand, well sprinkled with cement and fused in position. Aluminium is also used for lining concrete vessels. Contact between the metal and concrete is prevented by means of bitumen sheets or plastic laths, spaced at short intervals, the gauge of the aluminium being such as to withstand deformation in the spaces between the laths. A plaster lining of asphalt base, which can be applied with a brush and repaired by means of a blow-lamp, is now available, under the name of Gashell. Concrete vessels are shown in Figs. 136 and 139. These are lined on the Rostock and Borsari systems, respectively.

(543) Metals.

Metals were introduced on account of their durability and to provide vessels with an impervious surface which could easily be cleaned and sterilised. They have proved very satisfactory for fermenting vessels, but the change-over from the comparatively rough surface of wood was sometimes noted to have an adverse effect on fermentations. Reasons for this were mentioned in Section 538 but, in some cases, there were actual toxic effects on the yeast, due to solution of the metal (Section 546). This has, in many cases, been found to be due to impurities or lack of homogeneity in the metal and can be traced to electro-chemical action. In the case of non-toxic metals, corrosion which materially reduced the life of the vessels resulted from similar causes. These are now to a great extent avoided by improvements in metallurgy and experience in construction of the vessels. In many cases a film of oxide readily forms on the surface of metals in contact with moist air. This is generally hard and firmly adherent, so that the oxidised surface can be cleaned without damage, leaving a smooth, sterile surface that protects the metal from attack. The film on stainless steel and aluminium is invisible, while that on copper is black. The rust to which iron is liable is, however, among its serious disadvantages. Metals suffer from the disadvantage that they are excellent

FIG. 136. AIR-CONDITIONED FERMENTING CELLAR WITH CONCRETE VESSELS [illegible]

Facing page 762

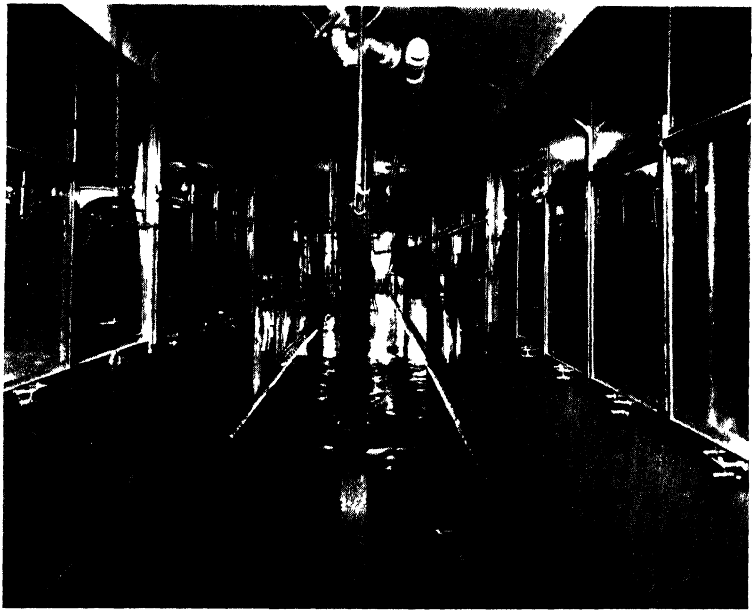

FIG 137

FERMENTING ROOM WITH VESSELS ENCLOSED FOR FULL AIR CIRCULATION
(ANSELLS BREWERY LTD, BIRMINGHAM)

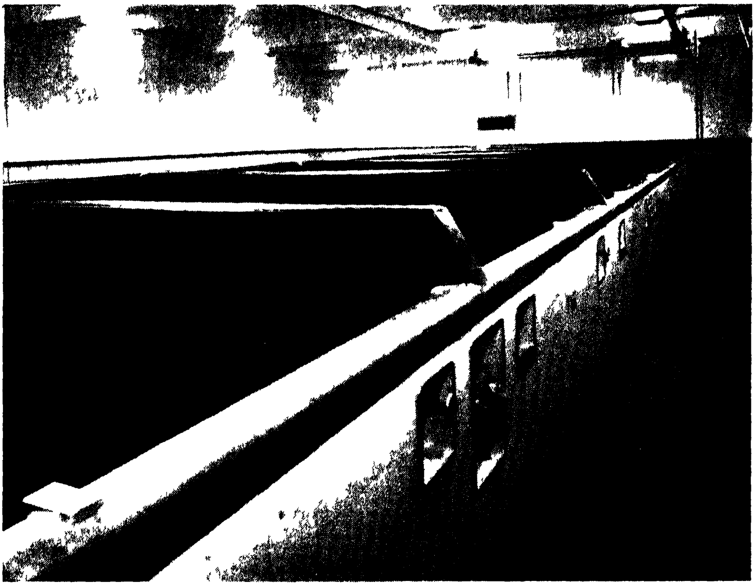

FIG 138

AIR CONDITIONED FERMENTING ROOM, WITH ENAMELLED COPPER VESSELS
(NORTHAMPTON BREWERY CO LTD, NORTHAMPTON)

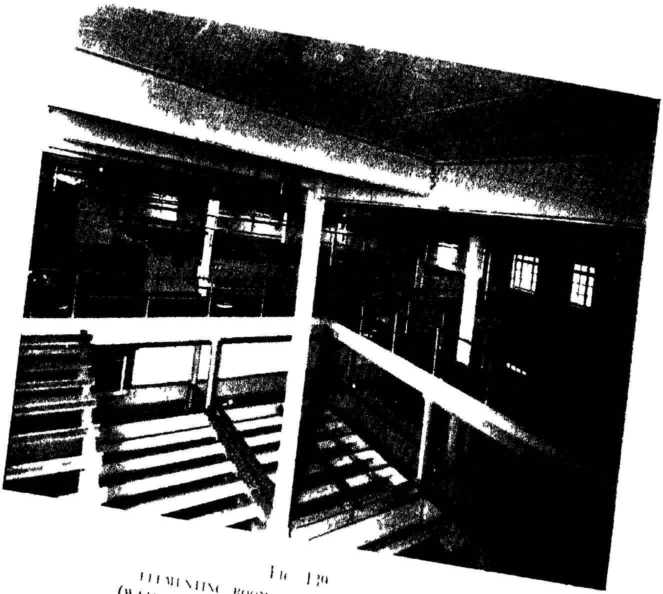

FIG. 139
FERMENTING ROOM WITH CONCRETE VESSELS
(WATNEY, COMBE REID AND CO LTD, MORTLAKE)

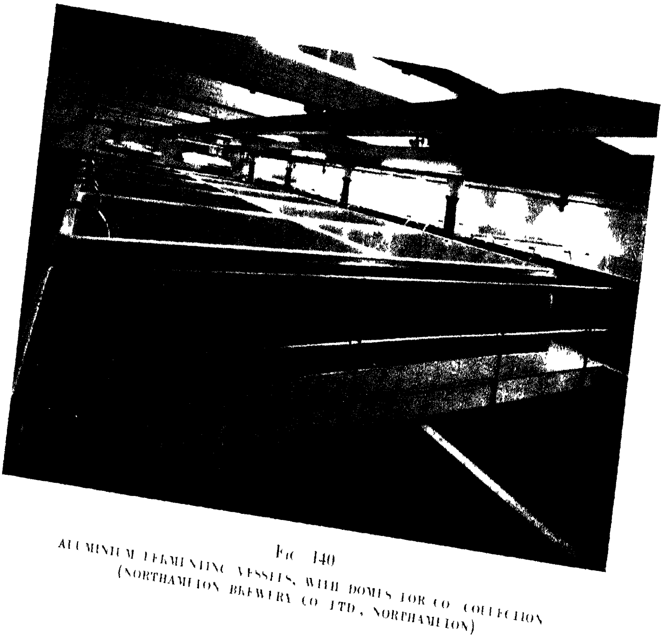

FIG. 140
ALUMINIUM FERMENTING VESSELS, WITH DOMES FOR CO_2 COLLECTION
(NORTHAMPTON BREWERY CO LTD, NORTHAMPTON)

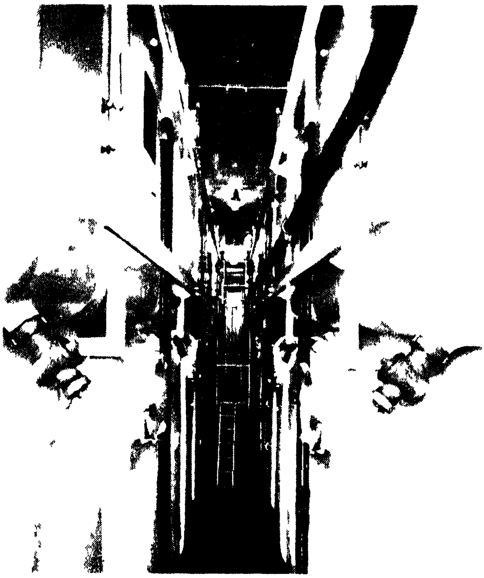

FIG. 141

ENCLOSED GLASS-LINED STEEL FERMENTING VESSEL FOR CO_2 COLLECTION. TRAVELLING STAGE FOR ACCESS TO [illegible] (BRASSERIES ARTOIS, LOUVAIN)

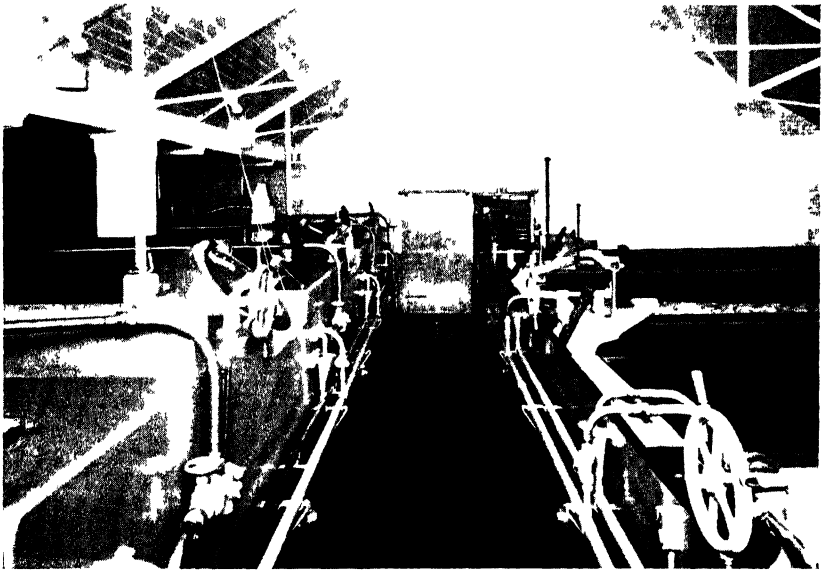

FIG. 142

CONCRETE FERMENTING VESSELS, OPEN AND ENCLOSED FOR CO_2 COLLECTION (HAMMERTON AND CO., LTD., LONDON) conductors of electricity. Hence, suitable steps are taken to insulate them and prevent the flow of small currents. Rapid heat transference may also be a disadvantage and many vessels have been lagged to prevent this, but the uncovered metal is used in modern constructions, reliance being placed on air as a heat insulator, with special care to control the temperature of the room and avoid draughts.

Spirit varnish and enamel linings are largely used with metal vessels. Mixtures of pitch and wax are very suitable, the Mammut linings being an example. These are washed with water, scale being removed with alkaline hypochlorite. Chloramine-T is a very suitable sterilising agent, 0·1 to 0·2% solutions being brushed on every time the vessels are used. This agent does not contain alkali and is not a detergent, but slowly gives off chlorine and sterilises the surfaces by liberation of oxygen from water. Gashell makes a good surface for metal vessels. It can be applied by means of a spray or brush, according as a thin or thick coating is required. Synthetic resins are used in a lining of American origin, known as Lithcote. It gives a glass-like surface, which is flexible and will withstand sterilisation by steam at any temperature not exceeding 250° F. Mammut is melted at about 300° F., is then applied hot and fused into the interior surface of the tanks.

(544) Iron and Steel.

The strength and cheapness of iron make it attractive as a possible material for the construction of fermenting vessels and storage tanks, but the readiness with which it rusts and is attacked by wort or beer prevents its use in an unlined state. It is, however, largely and successfully employed with such linings as have been mentioned. Care must be taken to avoid exposure of the iron through damage of the lining material, which is cleaned off and replaced or repaired as required.

Stainless steel is unique among metals suitable for the construction of fermenting vessels in respect of resistance to corrosive agencies. It has the disadvantage of high cost, due partly to the chromium used in the alloy and partly to manufacturing difficulties. "Stainless iron," or low carbon iron-chromium alloys, do not appear to have been used for fermenting vessels. They are comparatively soft and malleable, with a corrosion resistance similar to that of "stainless cutlery steel," but not so great as that of the true stainless steels or higher carbon steel alloys, containing other metals, such as tungsten and titanium, in addition to chromium and nickel. A typical composition is represented by 18/8/1, meaning that the alloy contains 18% of chromium, 8% of nickel and 1% of tungsten. These alloys are very ductile, highly-resistant, non-magnetic and can be dealt with *in situ* by oxy-acetylene or electric welding, without impairment of their corrosion-resisting properties. The latter are due to the formation of an invisible film of chromium oxide, on which is superimposed the direct resistance of nickel to corrosion. Nickel also appears to possess to some extent the power of forming a protective oxide film. Intergranular corrosion was formerly rather common at some little distance from welds in stainless steel, at points where the temperature of the metal had been raised to between 500° and 900° C., the temperature at the weld itself being much higher. The result of heat within this dangerous range of temperature was that the protecting films became discontinuous at the grain boundaries, and corrosion was easily set up between the grains. This liability to intergranular corrosion was overcome by modifications in the composition of some stainless steels. These should always be used for welded vessels.

Stainless steel has about three times the strength of copper and, consequently, a considerable saving of weight can be secured by using thinner sheets than are desirable with the latter metal. It can be cleaned in any ordinary manner, but the advisability of a high polish is debatable. for reasons already mentioned. Since corrosion-resisting properties are only required on the inner surfaces of brewery vessels, it is possible to secure the advantages of stainless steel at a much reduced cost by use of the stainless-faced material, known as Ingaclad. This is manufactured by casting mild steel in a mould round a welding quality of 18/8 Cr.Ni. type of stainless steel, followed by heat treatment of the composite ingot, to weld the mild and stainless steels together. This is made into ⅛ to ¾ inch plates, having a stainless facing about 20% of their total thickness.

(545) Glass-lined Steel.

The development of vitreous mixtures which, when fused on the surface of steel, cover it with a strongly adherent, smooth surfaced, corrosion-resisting coating of glass, with flexibility corresponding with the elastic limit of the steel, has placed "glass-lined steel" amongst the most suitable materials for fermenting vessels and tanks. Single-piece all-welded steel tanks are now made of great size and are used in preference to the older types built up from bolted rings. The completed shell is internally sand-blasted and evenly coated by spraying with a liquid mixture of the fusible materials. It is then dried with a forced circulation of hot air and placed in a preheated, gas-fired furnace, where it is subjected to a white heat, controlled by thermo-couples, for 40 to 60 minutes. The glass mixture is thereby fused into the surface of the steel, giving it a lustrous blue lining. Closed tanks are fitted with manhole doors and outside tightening valves, cocks, gauges, etc., with no internal threads. The fittings are finished bronze or chromium plated to give an attractive appearance to the tanks, which are generally painted white or cream.

Transport and erection of single-piece tanks, which may be as long as 40 feet, with a diameter of 10 or 11 feet, is clearly a delicate operation and would require extensive demolition of old buildings. Care must afterwards be exercised to avoid damage to the glass through rough treatment with ladders, boots, etc. Small cracks and pinholes can, however, be readily and efficiently repaired with a paste of dental enamel, which makes an extremely hard and resistant patch.

(546) Copper.

Copper is largely used in British top fermentation breweries for fermenting vessels, in many cases in the form of lining sheets riveted or welded together. Soldering which was originally used for copper linings is now abandoned. In newer constructions, copper is used as the material of the vessel and not as a lining. In many cases the copper is scoured and kept in a brilliantly polished condition, with no detrimental effect on the fermentations. In other breweries, the fermentations have suffered very considerably, in comparison with those in wooden vessels, on account of the toxicity to yeast of traces of copper dissolved in the wort. Attack on the copper is most obvious at the yeast level, where the green colour of basic copper carbonate and evidences of corrosion appear. It can also be detected by change in the colour of the metal exposed when the vessels are emptied. Under these conditions, yeast crops are frequently reduced, sometimes almost to vanishing point, and there is an increased amount of sedimentary yeast, with attenuations drifting further than their final figure in wooden vessels. Examined microscopically, the yeast appears granular, with many dead cells. Chapman[3] summarised the results of investigations in breweries where defective fermentations were experienced in copper vessels, as follows—

(1) Copper salts, when present to the extent of more than about 2 grains of metallic copper per gallon, seriously diminish the reproductive power of yeast, the results in practice being more noticeable when the vigour of the yeast is, owing to other causes, below the normal. Thus the poisoning effect of copper

salts will be, as a rule, most marked during the summer months, especially in those breweries where malts deficient in yeast-feeding nitrogenous substances are being used or where, for other reasons, the yeast is in a weakly condition.

(2) Very slight traces of copper, say less than 1 grain of metallic copper per gallon, do not appear to exert any adverse influence and there is some evidence to show that they may even act for a time as stimulants. It has been shown that yeast is capable of abstracting minute traces of copper from worts and that the poisoning effect is cumulative.

(3) A larger proportion of sedimentary yeast is formed in smooth-surfaced copper vessels than in wooden tuns, under similar fermenting conditions.

(4) The composition of the copper used, that is to say the nature and amounts of the various impurities present, has a considerable influence on the extent of the corrosion and on the rapidity with which it proceeds.

(5) A smooth, bronzed copper surface undergoes corrosion less readily than one which has been brightened and roughened by scouring with pumice or similar material.

The attack on copper at about the yeast line is due to the combined action of moisture, air and carbon dioxide. This results in the formation of basic copper carbonates which are dissolved in the wort to a certain extent. Analyses of dried yeasty matter, taken from this level after emptying the tun, gave 0·69% of copper on dry substance, equivalent to 4,830 grains per pound, while the pitching yeast from the same tun contained 1·54 grain per pound copper on dry matter, and the racked beer 0·22 grain per lb. Yeast from beers fermented in wood or glass-lined vessels may contain 0·14–0·21 grain per lb. of copper, or 20–30 parts per million, on dry matter.

It is consequently customary in many breweries to allow the copper to become coated with a resistant coating of oxide, giving the surface a bronzed appearance, and to clean it with a rough brush and caustic soda solution. Chapman found that the copper in the yeast deposited at the wort line was reduced from 0·69% to 0·16%, after the vessels had been treated three times in this manner, and was further reduced to 0·10% after they had been used three or four times more. In other breweries the surface of the polished copper is coated with China clay or enamel down to some little distance below the wort level. Lager brewers rarely use copper vessels on account of the danger of detrimental effects on fermentation, although many top fermentation brewers find no difficulty at all, even with brightly burnished copper.

(547) Aluminium.

Aluminium is very largely used for fermenting vessels and storage tanks. Its lightness and appearance, coupled with the possibilities of autogenous welding for erection and repair *in situ*, make it a very attractive material. Its comparative softness and liability to damage or deformation, the readiness with which it is attacked by alkalis or common salt and the need to guard against electro-chemical corrosion are its chief disadvantages, but these can be overcome by care in use and maintenance. It is employed as a lining for wood and concrete vessels and also without external casing, the gauge of the sheets depending on the service or pressures which the metal is required to withstand. Linings may be 1¼–2½ mm., solid vessels 3–4 mm. and conditioning tanks up to 8 mm. in thickness. Air of controlled temperature is circulated in the enclosed room below the gangway around the aluminium vessels in Fig. 140. The vessels are protected above it by fire-brick tiles between the steel supports. The tiles, shaped to provide a 1 in. air-space, are rendered with cement coated with asphalt, bonded to that of the gangway. Aluminium is very suitable, on account of its lightness, for yeast wagons and other small vessels which have to be carried about or moved on trolleys.

Attack by alkalis limits the range of possible detergents for use with aluminium, but 10 to 20% solutions of washing soda, to which 1 or 2% of water glass, sodium silicate, has been added, are without injurious effect and soften a scale of beerstone. The sides are best cleaned and polished with fine steel wool and a good grade soap in water. Removal of beerstone must be considered in connection with the influence of a rough surface on fermentation. Lager brewers generally hold that a slight thickness of scale is to be desired but, in top fermentation breweries, the danger of infection caused thereby is considered to outweigh any advantage of the roughened surface and beerstone is removed. Polishing with steel wool and soap is most effective for this purpose. Thick deposits of beerstone may, alternatively, be softened by application of the soda-silicate mixture or dissolved by nitric acid, diluted with three times its volume of water and made into a thin paste with kieselguhr. These are left on the scale for some hours and then brushed off. Nitric acid cannot be used in closed vessels, on account of the fumes, and must be prevented from access to valves and copper mains by stopping these with clay. Closed yeast vessels are cleaned by rubbing the wet surface with pumice stone in a circular manner.

The greatest safeguard against corrosion is secured by cleaning aluminium vessels immediately they are emptied and keeping them dry, when out of use. Corrosion rapidly sets in if water is allowed to lie in dents or hollows in the bottom of the vessel. Perfect drainage is consequently important. Many cases of pinhole corrosion occurred in the earlier days of the use of aluminium, but these are avoided by the employment of purer metal, containing not less than 99·3% of aluminium. It is usual to employ considerably purer metal than this, but its physical condition, depending on the treatment during production, is at least equally as important as purity. Electro-chemical corrosions, through contact with the ground or other metals, must be carefully guarded against. No water must be allowed to collect between the vessel and its external steel supports. Insulation from the earth is secured by means of bitumen, while attemperators, valves, etc., are equally carefully insulated from the aluminium. It is not advisable to use aluminium attemperators, as they are quickly corroded by the cooling liquor. Copper attemperators are, with advantage, slung, rather than joined to the sides of the vessels. The use of mercury thermometers is precluded, unless the bulb is steel-cased, as mercury from a broken thermometer rapidly pierces aluminium. It destroys the protective film and prevents its re-formation. Alcohol-filled thermometers are advisable. The practice of varnishing the inside of aluminium vessels increases the tendency to corrosion of the metal below the varnish, rather than preventing it. Rubber-soled boots should always be worn to prevent damage to vessels that it is necessary to enter and equal care must be taken with ladders.

(548) Fermenting Vessels for CO_2 Collection.

Methods of gas collection can be adapted to fermenting vessels made of any of the materials mentioned. Completely closed vessels are most satisfactory, but not always convenient. Domed aluminium covers, carried on runways above a row of vessels and adapted to be lowered at will over any of them until a seal is made with the head, are very efficient. An example of this arrangement with aluminium vessels is shown in Fig. 140. Fig. 142 is from a photograph of closed concrete vessels with Ebon linings. The domes in the former installation are about 2 feet in depth. An adequate seal is made by lowering them until they dip into the yeast head, when the space above the head to the top of the sides of the vessel is fairly deep. A space of about a foot is allowed round the dome on three sides of the vessel, with a rather wider space on the other, to permit of sampling. CO_2 collection is dealt with in Volume III.

(549) Attemperators.

Most fermenting vessels are fitted with attemperating coils of copper or heavily tinned copper. The pipes are frequently circular in section, but a flattened shape has greater cooling efficiency. An improved design by Watkins is shown in Fig. 143. The flattened tubes are fitted at their ends into curved girders and supported by pivots at each end of the fermenting vessel. The whole can be rotated through 180°, so that the convex face may be upwards or downwards, according to the volume of wort in the vessel, or vertically for cleaning purposes.

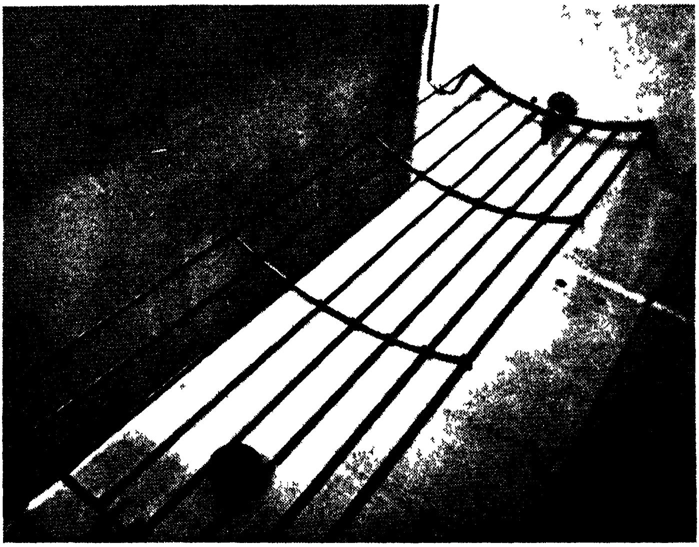

FIG. 143
ROTATABLE ATTEMPERATOR (WATKINS)

It can also be raised or lowered, to be always near the surface. Copper attemperator pipes are always carefully insulated from contact with any other metal, particularly aluminium, so that electrical action shall be reduced to the minimum. This might, otherwise, increase the possibility of attack by wort, which is only temporarily prevented by tinning and may be increased when the tin begins to wear off. The solution of copper is, however, extremely small in any case. Koch [4] found quantities of copper amounting to about 0 5 parts per million in wort fermented in wooden vessels with copper attemperators, but this was reduced to 0·1 or 0·2 parts per million in the beer, the greater quantity being deposited in the yeast or storage tank sediments. In some cases, the attemperating liquor is allowed to flow into open funnels on drain pipes. This affords a place where micro-organisms may develop, but has one possible advantage in that pinholes in the attemperator pipes can be detected by discoloration of outflow water by beer. If used, they should be covered with sight glasses. Attemperators are most efficient when close to the surface of the wort.

STORAGE CELLARS

(550) Construction of Cellars.

It is usually necessary to maintain a temperature between 32° and 36° F. in lager cellars but, in some instances, this is reduced to 28° to increase the stability of export beers. The insulation of the walls, floor and roof must, consequently, be very efficient in order to keep the expenditure of refrigeration as low as possible. The cellars are also restricted in size, so far as is consistent with convenience, to minimise calls on refrigeration when tanks are washed or filled with beer. For this reason a number of cellars are frequently constructed with their length at right angles to a transverse outer cellar, communicating with the exterior. Storage capacity can, with this arrangement, be expanded or reduced with economy in refrigeration. In many breweries, where site and drainage permit, underground cellarage is constructed with advantage, but it is necessary to avoid reaching water levels and the cellars must, in others, be above ground. The cost of land has made tall buildings increasingly necessary and these have proved well suited to brewery purposes, the "stock houses" of many American breweries being cases in point. They may contain, from the lowest floor upwards, 4 or 5 stories of storage tanks, fermenting rooms, refrigerator with hop store on the same floor, and "cool-ship" on the top. Substitution of buildings of several floors in place of underground cellars has, in most cases, not proved uneconomical in refrigeration, because the insulation of underground cellars, particularly in damp ground, is not so simple as it might appear.

Refrigeration is still usually carried out by direct ammonia expansion or brine coil systems, frost being thawed off the pipes every 14 days or so. The newer methods of air-conditioning with unit coolers or systems to supply a number of cellars with air at fixed temperature and humidity are, however, being increasingly adopted. Cleanliness is greatly facilitated by keeping the air as dry as possible. Air filtration is also desirable to avoid infection of the interior of empty vessels or mould growths on the walls and tanks.

Floors insulated with cork, covered with asphalt, will not bear the weight of tanks. Hard wood blocks are consequently placed under their feet to give a bearing right on to the concrete below. These are floated over with one inch of asphalt, carried over the floor and six inches up the walls. Granolithic flooring is harder wearing, but is liable to crack in cold rooms over cork, where rock asphalt, embodying sharp sand or granite chips and kept sufficiently plastic, is preferable. All washing-out of tanks should be through the outflow cock and thence by fixed mains through the walls to the nearest drain.

(551) Storage Tanks.

Wooden casks of comparatively small capacity, ranging from 20 to 60 barrels, were, at one time, universally employed and still are in many breweries, where their advantages are appreciated, despite the great economies in space and labour secured by the adoption of larger, cylindrical tanks of metal and the still greater saving in space with rectangular concrete vessels, which can form part of the structure of the cellars. The wooden casks are specially constructed to withstand the pressure developed during continuance of fermentation, with ends arched inwards and strengthened by connecting bars and bolts. They are lined with pitch to prevent adsorption of beer, adhesion of yeast and sedimentary matter and escape of CO_2 through the pores. The annual removal of storage casks from the cellars for repitching is arduous and necessitates suitable space and hatchways. This, with the great space required for storage of a given quantity of beer in casks of 20, 40 or 60 barrels capacity, has been decisive in the adoption of larger metal tanks in practically all modern installations, although wooden vats of five or six hundred barrels capacity are made to withstand the necessary pressure and are still widely used in American breweries. These are upright vats, usually of cypress or Californian redwood, lined with pitch or Mammut. The practice of vatting top fermentation ales and stouts is now restricted to a few special beers. An example of oak storage vats of 19,000 gallons capacity is given in Fig. 148.

Any metal liable to corrosion by beer or moist air must obviously be avoided in the construction of tanks, unless its interior is completely lined with some resistant material, not liable to crack, flake or rub off too quickly. Iron tanks, lined with pitch, Lithcote, etc., are used, but have the drawback that the lining must be carefully watched and periodically renewed, as with wooden vessels.

Glass-lined steel tanks are probably more generally employed than any other type. Their strength is sufficient to permit of support of very long horizontal tanks at the ends only. The load can consequently be placed direct on beams or girders. Floors need not be designed to carry them and are, in many cases, entirely omitted when several tiers of tanks are installed. Access to these is then gained from galleries. In two-tier constructions, the upper tank can be supported on the extended legs of the lower or it may be suspended at the ends from beams or brackets on the columns. The tanks in some breweries are walled in so that only the fronts project into the working space. In general the glass-enamelled surfaces wear exceedingly well and no damage need be apprehended, if reasonable care is taken and rubber-soled boots or mats are used when it is necessary to enter the tanks. The very smooth surface, to which yeast does not strongly adhere, makes cleaning a comparatively simple matter. Several tanks can be mounted one above another, with an intervening concrete bedding, in the latest "column" construction (Fig. 147).

Aluminium is extensively adopted and makes excellent tanks, although great care must be exercised in their upkeep and cleaning, in order to avoid the corrosion and pinholes to which this metal is liable, though this danger has been to a large extent overcome by the use of pure metal and improved fabrication. The lightness of aluminium makes it possible easily to erect two or three tiers of tanks, the upper having about two-thirds the capacity of the lower. When aluminium tanks are saddled, care is taken to avoid risks of corrosion through electric currents by insulation of the tanks from the supports.

Stainless steel has not been in use for long but, provided the metal is suitable and erection has been properly carried out, with the necessary care to avoid damage in its resisting properties at welded joints, it would seem to be the ideal material for tank construction. Tanks of 50 barrels capacity have been installed in a few breweries, but their cost is almost prohibitive for general use at the present time. This is greatly reduced by the use of Ingaclad (Section 544).

Rectangular ferro-concrete tanks lined as previously described are made of very large capacity and with dimensions that most conveniently suit the building. The temperature of the beer is controlled by internal coils of aluminium or tinned copper through which a non-corrosive brine or 20% solution of denatured alcohol is circulated. These tanks have proved very satisfactory and are now extensively used.

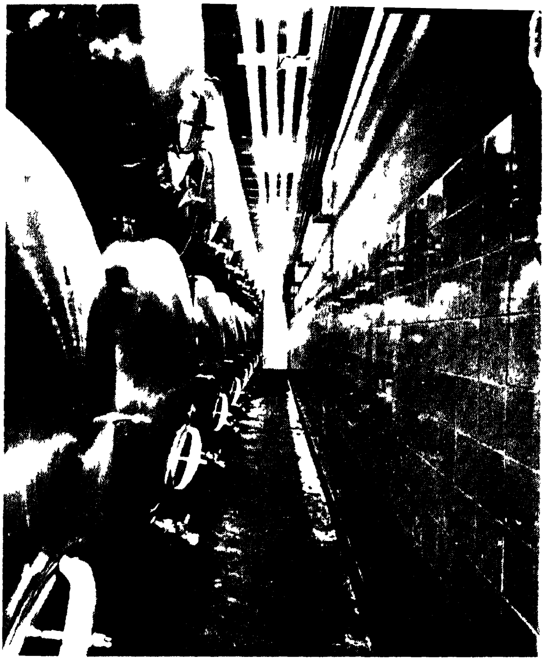

FIG. 144
STAINLESS STEEL STORAGE TANKS 50 HLS. C
(TUBORG BREWERY, COPENHAGEN)

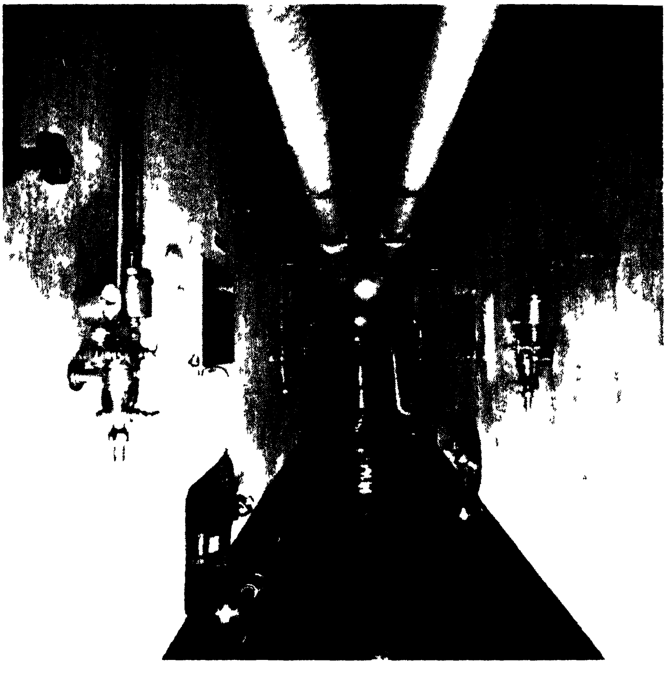

FIG. 145
CONCRETE STORAGE TANKS (BRASSERIE C. KLEINKNECHT, STRASBOURG)

[Facing page 77.

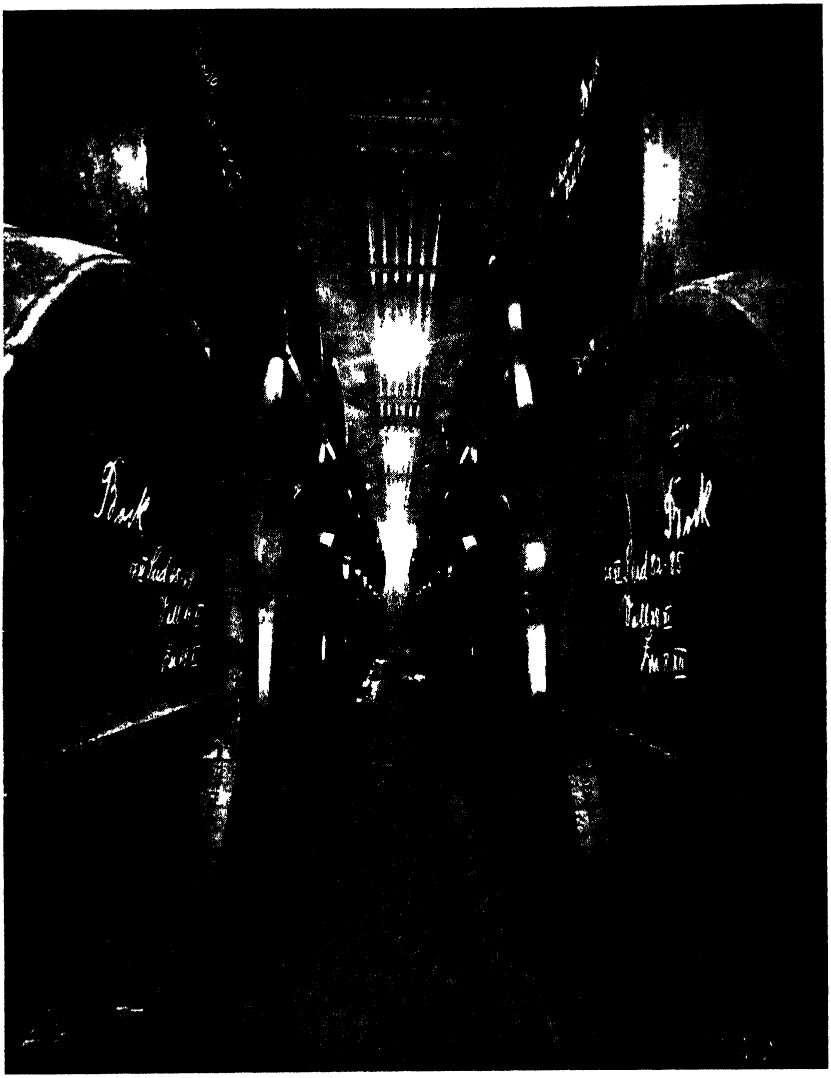

FIG. 146

OLD STYLE LAGER CELLAR, WITH OAK VATS
(LUZERNER BRAUEREI ZUM EICHHOF A. G., LUCERNE)

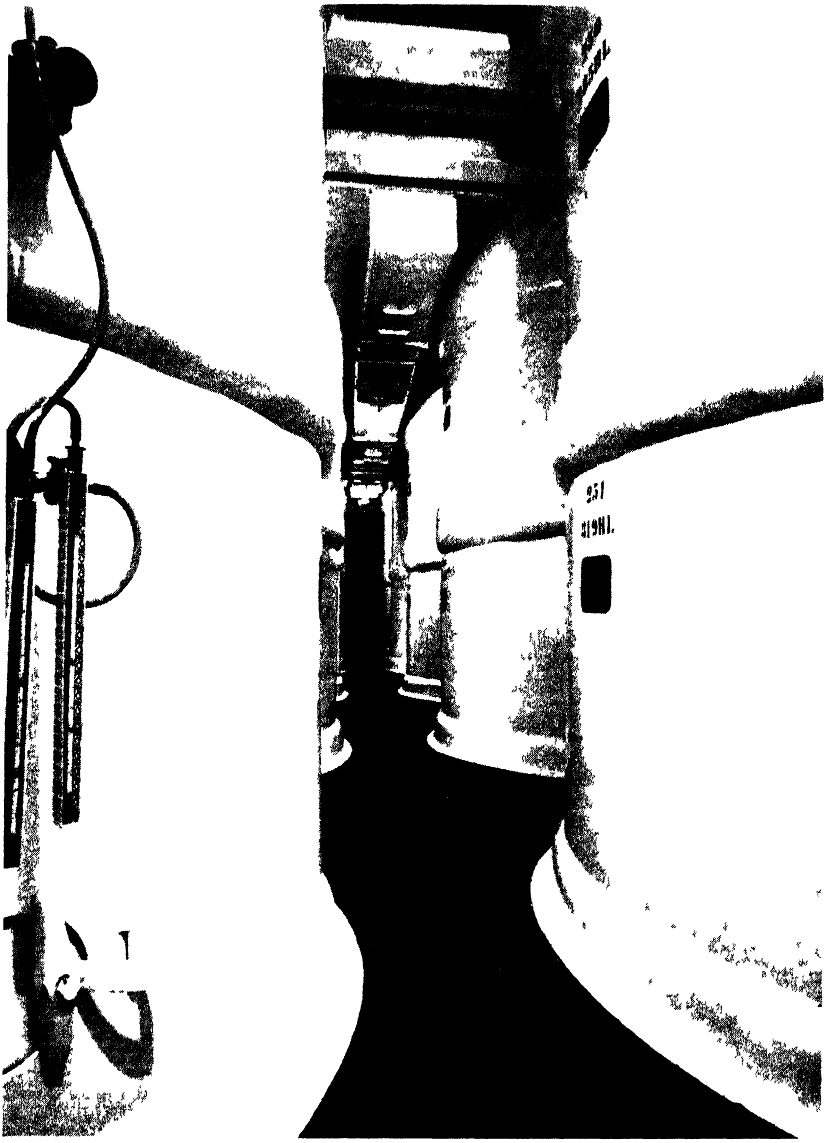

FIG. 147

MODERN AIR CONDITIONED LAGER CELLAR WITH GLASS-LINED STEEL COLUMN TANKS
(BRASSERIES ARTOIS, LOUVAIN)

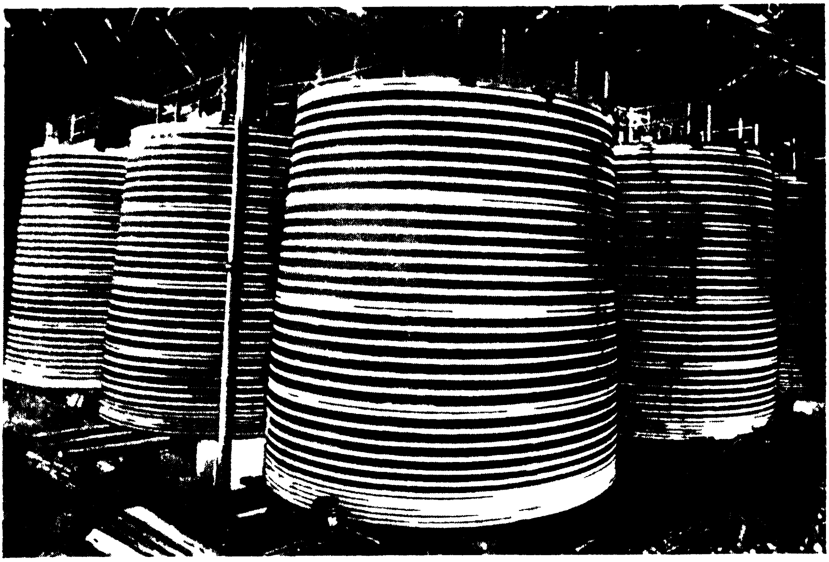

FIG. 148

OAK STORAGE VATS

(ARTHUR GUINNESS SON AND CO. LTD. DUBLIN

(552) Size and Surface of Storage Tanks.

The capacity of a cellar and economy in refrigeration are increased by the use of larger tanks, but their size is governed, in the first place, by the output of the brewery and the quantity of beer that can be disposed of in one day. It does not necessarily depend on the length of the brews, since it is common practice to divide and blend several successive brews in a number of tanks. Tank capacity has, in addition, a material influence on the course of fermentation and duration of maturation. Increase diminishes the rate of cooling in tanks of any one material and this, with reduction in the rate of yeast settlement, leads to greater attenuation in lager breweries. Horizontal tanks are more generally used than vertical, unless the capacity is quite moderate. Very large vertical tanks have disadvantages, arising from unequal cooling and formation of layers of beer at different temperatures. This leads to greater circulation.

The nature of the inner surfaces of tanks also has a considerable influence on the course of fermentation and maturation, although experience with tanks of different materials appears to show that the quality of the beer depends more on correct temperature control. The pitched surface of wooden casks has proved to be admirably suited to yeast sedimentation and clarification of beer. Glass-lined steel is not quite smooth, satisfactory sedimentation and clarification are secured, but the surface is not quite so good in this respect as pitch. The sediment does not settle so firmly on smooth surfaces and is liable to be carried to the filters with the beer. Some lager brewers, who have both wooden storage casks and tanks, reserve the former for their best beers.

The capacity for heat transference of different materials must be considered in relation with refrigeration of the cellars and in respect of the effects of rapid and slow cooling on the beers. It is low for wood and consequently the cellars can be kept at lower temperatures than with metal tanks. Beer in wood is also less liable to be affected by fluctuations in cellar temperature. A too rapid and intense cooling is detrimental to maturation on account of its restrictive influence on yeast activity, but the greater capacity of metal tanks tends to counterbalance this defect. Internal cooling is more economical than cellar cooling. It is readily controllable, but care has to be taken in order to avoid damage to the yeast and check to fermentation. It is independent of cellar temperature and particularly applicable to concrete vessels, which would otherwise be unsuitable for storage tanks, on account of the slow heat transfer through their thick walls.

(553) Fittings of Storage Tanks.

Storage tanks are fitted with manhole doors to permit of entrance for cleaning, with cocks to drain out the washing liquor and for filling with beer and racking. The filling and racking cock is placed as near to the bottom as possible, without danger of sucking out the sediment, and is constructed in such a way as to avoid any accumulation of yeast. A small sample tap is also provided at about half the height of the tank. Thermometers are placed in convenient position for observation and pressure regulating or bunging apparatus, of which several types are in common use, are fitted on the top of the tanks, with pressure gauges and, sometimes, safety valves. Each tank may be fitted with its own automatic pressure regulator or a number may be united to a single liquid column. The latter is a simple and convenient arrangement and usually consists of a vertical tube, filled with water to such a depth that the hydrostatic pressure balances the CO_2 pressure in the tank. The gas connection is made through a narrow tube, leading from the gas outflow cock on the top of the tank to the bottom of the water column, as in Fig. 149. The small flask shown in this sketch is intended to retain foam carried over by the gas.

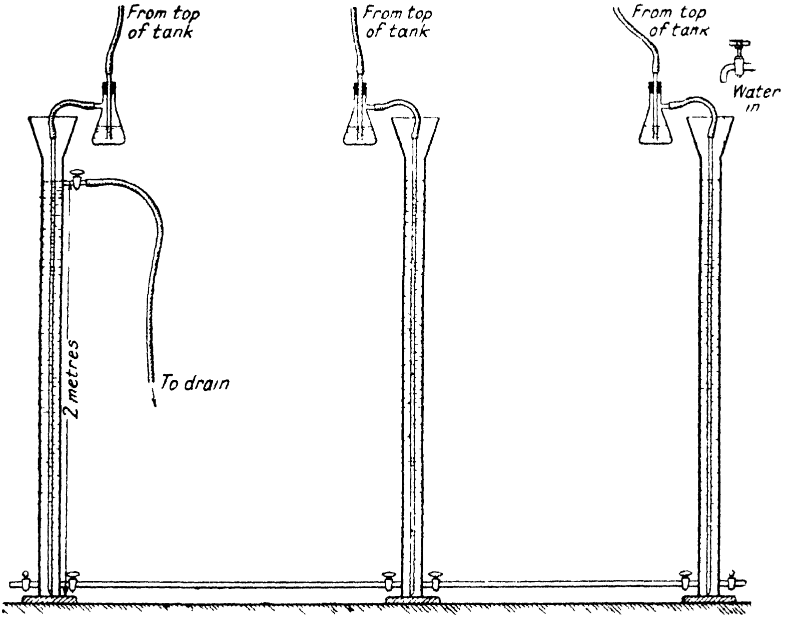

Fig. 149

WATER COLUMN PRESSURE REGULATOR

The pressure regulators attached to individual tanks may consist simply of a U-tube containing mercury to act as a manometer, the quantity of mercury being adjusted to the pressure required. A simple, but very efficient bunging apparatus for single tanks is shown in Fig. 147. In other installations a lever regulating pressure valve is fitted on the top of the tank. Water column pressure regulators and pumps for circulating the refrigerated 20% alcohol are used with many concrete storage tanks.

(554) Summary.

Modern fermenting rooms are designed on the most hygienic principles. They are air-conditioned, their walls and ceilings are of waterproof, washable materials, while mains, beams and other possible lodgments for dust or condensed water are eliminated as far as possible and not allowed to run over the vessels. The latter are usually of metal or concrete, with smooth, unattackable linings. Many brewers, however, still prefer wood which, they claim, has many advantages, when in sound condition, over metals or any other available material. Among these are a low coefficient of heat conductivity, and a surface which is sufficiently smooth to permit of perfect cleaning and yet better adapted to fermentation than the smoother surfaces of metals, glass or enamel. The same preference for wood, though in this case pitch lined, is shown by many lager brewers for their storage vats. This is partly due to their necessarily limited size, since the greater surface presented to the beer by several lager casks than by one large tank facilitates clarification. Slate has its adherents, but the tendency is to replace it by aluminium or stainless steel. Almost any type of vessel can be adapted to the collection of CO_2, either by complete enclosure or by the use of a dome.

The old type of storage cellar, with direct expansion or brine coils for refrigeration, is giving place to air-conditioned cellars, on account of the advantages of dry air and cleanliness secured thereby. The contrast between an old and modern cellar is illustrated by Figs. 146 and 147. There is the same tendency towards the elimination of wood and the greater biological security of metals or lined concrete as is seen in fermenting vessels. The advantage of comparatively small cellars, in which temperature changes due to frequent cleaning and filling of tanks with uncooled beer are avoided, is generally recognised. Air conditioning also avoids filling the tanks with infected air each time they are emptied.

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CHAPTER XXXII

PRINCIPLES OF FERMENTATION

TYPES OF YEAST

(555) Brewery Fermentations.

Fermentation is carried out in breweries in two very distinct ways. These are known as top and bottom fermentation processes, according as most of the yeast rises to the surface of the wort or sinks to the bottom of the fermenting vessel when its multiplication is completed. The guiding principles are the same in both, the chief differences being in the nature of the yeast, the temperatures adopted and the manner in which the " yeast crop " is removed from the beer. The first stage of active fermentation and the second or cleansing stage, in which the yeast heads are removed, are managed in several ways in top fermentation breweries. These are known as fermentation systems, two of which, the Burton Union system and the Yorkshire Stone Square systems, are still characteristic of the districts in which they were developed. In bottom fermentation breweries, the beer is run down from the fermenting vessels, leaving a firm sediment of yeast. In whatever way the fermentation is conducted, the fact that on its successful issue depends not only the alcohol content of the beer but also, in large measure, its flavour and stability, is never lost sight of. The aim is always to mix a healthy pitching yeast with the wort, under conditions that will ensure a rapid commencement of its growth, so that other micro-organisms shall have little chance to gain a footing in the wort. Since the pitching yeast must be collected from one fermentation for use in another, it is equally necessary to protect it from infection and only save yeast that is uncontaminated. To this end, yeast that separates first and is contaminated with hop resins and protein matter, and that which separates last and forms a protecting cover for the wort, is rejected. The middle crops only are used again, whether they be the middle heads of top fermentations or the middle layers of sedimentary bottom yeast, on which the surface-protecting layer settles when the beer is run down.

(556) Flocculation and Attenuating Power of Yeasts.

Different races of yeasts, whether they be pure cultures or constituents of an ordinary mixed pitching yeast, can be distinguished by the rate at which they ferment or the degree to which they will attenuate wort under normal conditions. The latter does not imply that the yeasts actually have different attenuating powers, since they will ferment wort to the same extent, if kept in suspension by continuous shaking, as in the experimental method for determining limiting attenuation. This is a function of the composition of the wort and not of the yeasts themselves. The variations noticed in practice do not depend on greater or less ability to ferment this or that constituent of the wort, dextrins for example. Active fermentation only proceeds while yeast remains suspended in the wort. A yeast that tends to separate rapidly from the wort, either by rising to the surface or by settling to the bottom of the vessel, consequently acts as if it were a weakly attenuating yeast and is usually described as such. Another that shows little tendency to separate from the wort behaves as a strongly attenuating yeast.

The difference depends on the flocculating properties of the yeasts. Some tend to flocculate in the wort and the rapid stream of gas bubbles evolved in top fermentations brings the small clumps to the surface. Fermentation would consequently cease unless the heads so formed were roused back again. Others remain suspended in a more discrete or finely divided state throughout the fermentation and fully attenuate the wort, with little or no rousing. There is thus a reciprocal relation between flocculation and attenuation. This is exemplified by two types of yeast in British Breweries, which are often described as fast and slow yeasts. They are characteristic of certain fermentation systems and localities, after which the types are also frequently named. The Burton yeast is a fast type, and usually non-flocculating, while the Yorkshire yeast ferments more slowly and requires a good deal of rousing to keep it in the wort. The latter is, consequently, a weakly attenuating yeast in practice. Attenuation is usually carried by it to a racking gravity one-third or one-quarter of the original gravity. The beers are characteristically full flavoured and carry a good condition. Their stability tends to be lower than that of beers attenuated to one-quarter or one-fifth by the faster, more strongly attenuating yeasts, on account of the comparatively large proportion of fermentable matter remaining at racking. London and Scottish yeasts generally have intermediate properties. Variations in the rate of attenuation also occur among yeasts of the flocculating and non-flocculating types.

Similar differences characterise the two types of bottom fermentation yeasts, named after the weakly attenuating Saaz and strongly attenuating Frohberg yeasts (Section 641). The former flocculate readily and are known as " break " yeasts, while the latter, " dusty " or " powder " yeasts, remain suspended in the worts. The latter are preferred for beers that must be fully attenuated and very stable in trade.

It is not yet known how far the flocculating property can be attributed to racial characteristics or to variations in cultural conditions. Schönfeld [1] noticed that a pure yeast from the brewery culture apparatus often breaks badly and attenuates very fully in the first fermentation, but regains its flocculating property in subsequent brews. He also showed that both flocculating and powder yeasts might be obtained from one single cell culture and were capable of transmitting these properties on further cultivation. Yeast cells are so small that it might be expected that conditions that favour the coagulation of colloidal particles would have something to do with the flocculation of yeast. McCandlish and Hagues [2] noticed than an increase in the hydrogen ion concentration of the wort caused a certain yeast to flocculate, but changes in p_H value, within the normal range, do not appear to provide the explanation of flocculation (Silbereisen [3]). It is possible that this will be found to be associated with diffusion from the cells of some substance that can act as a flocculating agent. Stockhausen and Silbereisen [4] obtained evidence that a substance of the nature of yeast gum could function in this way.

(557) Tests for the Flocculating Power of Yeasts.

It is possible to obtain an idea of the probable behaviour and attenuating power of yeasts by ascertaining the rate at which they settle in water. Schönfeld [5] found that strongly attenuating yeasts usually gave a milky suspension in water, while those that break readily in practice generally form flocks in water, though there were exceptions. Only the most strongly flocculating top fermentation yeasts give a decided separation when mixed with distilled water at the rate of about 3%. Burns [6] suggested the following rapid test for " clumping power " : 1¼ oz. of pressed or liquid yeast is mixed with half a pint of tap water and poured into a cylinder. A good clumping yeast flocculates immediately, in the case of a liquid yeast, and in about a minute with pressed yeast. After 5 or 10 minutes, when the yeast has settled, the opacity of the supernatant liquid is noted. The stronger the clumping power, the more complete is the separation. Allowance must be made according as liquid or pressed yeast is used, because the barm ale in liquid yeast is a powerful flocculating agent. A good flocculation with pressed yeast would indicate only rather weak clumping power with liquid yeast.

A more accurate test is carried out by mixing liquid yeast with about twice its volume of distilled water, filtering with suction on a Buchner funnel and washing once with a little distilled water. 5 grams of the cake so obtained or 5 grams of brewery pressed yeast are mixed with 100 ml. of distilled water. The yeast only flocculates at this stage if its clumping power is very great. 10 ml. of the suspension are placed in a 15-ml. graduated, tapering centrifuge tube. 1 ml. of the sodium acetate-acetic acid buffer, p_H 4·6, used for determination of the diastatic activity of malt, is added. The tube is shaken vigorously for a few seconds, then tapped to dislodge yeast flocks from the froth. After standing 10 minutes the volume of the settled, flocculated yeast is read off. If the volume is over 1 ml. the yeast is of strong clumping power, if less than 0·5 ml. the yeast is likely to show weakness of flocculating power in fermentation. A numerical value for the clumping power of yeasts can thus be obtained and this gives a good guide to their attenuating power in the brewery.

(558) Segregation of Yeast Types.

A striking difference will be noticed in the manner in which suspended and flocculating top and bottom yeasts separate from the wort. The suspended yeast in a top fermentation consists, to a preponderating extent, of cells that will ultimately settle to the bottom of the vessels. That is to say the sediment yeast in a top fermentation has more strongly attenuating powers than that which forms the head. In bottom fermentation, both yeasts ultimately settle to the bottom of the vessel, but the weakly attenuating yeast settles more rapidly and corresponds with the rising yeast of top fermentations, while the highly attenuating suspended yeast corresponds with the sedimentary yeast of a top fermentation.

It is probable that all pitching yeasts, unless they are pure cultures, are mixtures of at least two types. A partial separation of strongly and weakly attenuating bottom yeasts can be effected by pumping wort from one fermenting vessel to another, immediately after the first heads have fallen. This results in transfer of the suspended yeast, while that which has already settled out remains behind. A very complete segregation of the two types can be effected by repeating this operation several times. Bishop and Whitley[7] showed that a similar segregation of weakly and strongly attenuating types of top yeast could be secured by separating the top yeast from that which settled to the bottom of the vessel. If the latter is contaminated with sedimentary matter, its attenuating power is reduced for reasons detailed in the next Section, so that a dropping system which leaves behind the sediment, but carries forward both yeasts, ultimately leads to a separation of types with more distinct differences in attenuating powers. These points are illustrated by the results of experimental fermentations of wort of 1050 sp. gr. by yeasts from different breweries, Table 219. Such striking differences in racking gravity as are there shown would not occur in practice, since the flocculating yeasts would be roused into the worts. In the experimental fermentations they outcropped rapidly and the fermentations were not stirred.

TABLE 219.—RELATION BETWEEN ATTENUATION AND SEDIMENTATION OF TOP FERMENTATION YEASTS (BISHOP AND WHITLEY)

Yeast	A	C	D	E
Racking gravity	1032·6	1020·2	1020·1	1010·7
Sedimentary yeasts gram/litre ..	1·6	2·6	2·3	4·4
Yeast in suspension ,, ...	0·1	0·6	0·8	0·4
Top yeast ,, ..	14·2	21·2	20·4	17·7
Total yeast ,, ..	15·9	24·4	23·5	22·5
Limiting attenuation	1007·7	1007·2	1007·1	1007·1

The relation between suspension and attenuation is well shown by the quantities in suspension at different times of the experimental fermentations with the weakly and strongly attenuating yeasts, A and E in Table 220. The most striking difference occurred between 18 and 42 hours. The average weights of yeast in suspension during this period were 4 grams per litre with A, and 8·2 grams per litre with E. Almost the whole of the difference in attenuation depended on the 10 or 12 hours during which E remained in suspension and multiplied, while A was coming out. Curves showing the fall in gravity correspond closely with those showing the quantity of yeast in suspension. Another point noticed in these experiments is suggestive when considered in relation with brewery fermentations. This was that E skimmed bright, but A, having attenuated the wort very incompletely, continued to grow and threw small crops with a slowly falling gravity.

TABLE 220.—YEAST IN SUSPENSION AND OUTCROPS WITH WEAKLY AND STRONGLY ATTENUATING YEASTS (BISHOP AND WHITLEY)

Hours	0	18	30	42	66	90	162
Yeast A.							
Wort gravity ..	1050·5	1047	1041	1038	1035	1034	1032
Yeast in suspension gram/litre	4·15	5·4	4·1	2·6	1·5	1·3	0·8
Total yeast skimmed „	0	0	4·1	8·1	—	—	12·8
Yeast E.							
Wort gravity ..	1050·5	1043	1030	1012	1011	1010·5	1010
Yeast in suspension gram/litre	4·15	6·7	10	7·8	1	0·7	0·5
Total yeast skimmed „	0	0	0·2	10·1	19·2	19·2	19·2

Fermentations with a slow or Yorkshire type of yeast, under whatever system they may be conducted, exemplify this. As soon as the periodical rousing is stopped, the yeast separates and attenuation comes practically to an end. If this is too early, the beer will rack with too much fermentable matter and be liable to develop excessive condition in cask. Further rousing before skimming is required to prevent this.

(559) Persistence of Type in Yeasts.

Despite the number of races which may occur in pitching yeasts, these remain remarkably constant in their characteristics through many fermentations in some breweries. It may be assumed that the practice of using the middle heads of top fermentations and the middle layers of bottom yeasts effects a kind of natural selection, which tends to uniformity by eliminating races unsuited to the cultural conditions or differing widely from the average, when the bulk of the yeast consists to a preponderating extent of one type. On the other hand, the mixture of types explains some of the changes in attenuating power of successive generations of pitching yeast in other breweries. If the yeast consisted of types which differed markedly in attenuating power, use of those which rose most readily into the heads would tend to a continual decrease in the attenuating power of the pitching yeast. The decline in attenuating power that becomes marked in a few fermentations in some breweries may be attributed to this, in many cases. Wort composition and other environmental conditions may contribute to the change or even be decisive in causing rapid reversion to the normal fermentations of a particular brewery, after a change of yeast has been obtained with the object of increasing attenuation or eliminating some defect. Bishop and Whitley suggested that segregation of sedimentary and outcrop yeasts may account for the gradual decline of attenuating activity when purified yeast obtained by the method described in Section 575 is used in the brewery. The original culture would no doubt include both sedimentary and outcropping types. The sedimentary yeast is preserved when the whole fermentation is transferred from smaller to larger bulks of wort in the propagating vessels, but is gradually eliminated when the normal practice of using the outcrops only for pitching is resumed in the brewery.

YEAST GROWTH AND FERMENTATION

(560) Phases in Yeast Growth and Fermentation.

The diagram in Fig. 150, representing the changes in temperature, specific gravity and p_H value during a brewery fermentation,

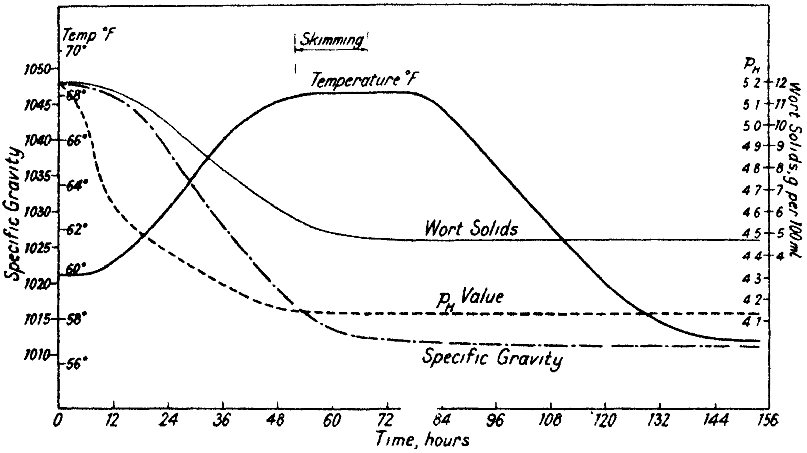

FIG. 150

DIAGRAM OF BREWERY TOP FERMENTATION, SKIMMING SYSTEM

The curves represent specific gravity, temperature and p_H value plotted against time.

skimming system, shows three distinct phases in the curve for specific gravity, plotted against time. These correspond fairly closely with three phases in yeast growth. (1) *A lag phase* before multiplication commences during which there is practically no fall in specific gravity. This gradually merges into a steeply descending, almost straight portion of the curve representing (2) *the logarithmic growth phase,* in which the number of cells is doubled in equal intervals of time, one cell producing two cells which grow and then produce two buds and so on in each generation. This lasts for about 24 hours, after which the curve bends outwards in (3) *the phase of restricted growth,* in which accumulation of the products of fermentation gradually retard and finally stop growth.

The three phases of yeast growth have great significance in brewery fermentations. Some time is required for the cells of the pitching yeast to start growing, and this lag phase may be so prolonged as to cause anxiety, owing to the opportunity offered for the development of other organisms in the absence of rapid yeast growth. The lag phase can be greatly shortened or an immediate commencement of fermentation assured by pitching with yeast which has been mixed with strong wort at about 70° F. and is actively working when required.

Competing organisms have little chance of development during the following period of rapid growth, but stimulation of the fermentation by rousing and, particularly, by rise of temperature is desirable. Slator[8] found that the rate of fermentation was doubled by a rise of temperature of 9° between 59° and 68° F., which represents the range commonly adopted in top fermentations. Top yeasts, however, ferment most rapidly at about 95° F. and bottom yeasts at about 77° F., fermentation being arrested at about 122°. These high temperatures would be most unsuitable for brewing, since they impair the vitality of the yeast and stimulate the development of bacteria. The health, purity and vigour of the newly formed yeast and the flavour of the beer are much more important than great rapidity of fermentation. On the other hand, a very slow fermentation at too low a temperature also acts adversely on the yeast and, by checking its activity, gives greater opportunity for wild yeast growth. The best results are obtained by allowing a free course to the natural rise of temperature, due to decomposition of fermentable sugars, up to about 68° or 70° F. with top fermentation beers, the higher temperature only with stronger beers, and to about 48° or 50° F. in bottom fermentations.

During this period of rising temperature, which is normally at a rate of about 1° F. in 8 hours in top fermentations and must occasionally be slightly checked to prevent the maximum being exceeded, there is a rapid evolution of CO_2 with formation of rather light, rocky heads, which differ greatly in quantity in the slow bottom and rapid top fermentations. Dark coloured sedimentary matter and hop resins are brought up and form patches on the yeast heads. These must be removed, without unnecessary loss of yeast, in order to prevent their redispersal in the wort, with detrimental effects on the flavour of the beer. The lighter heads fall back when growth slackens and, soon after, the yeast commences to rise in the form of more solid heads. Falling back of the first heads marks the period at which the wort is transferred to cleansing vessels in some fermentation systems. The maximum temperature is reached at about 50 hours in Fig. 150, at which time skimming commences. This is generally rather before reproduction ceases. Attenuation practically ceases at about 76 hours, and the temperature is then gradually reduced to that desired at racking.

There has been a considerable amount of discussion on the restricting influence of CO_2 and alcohol on yeast growth. Slator's experiments with sugar solutions saturated with pure CO_2 indicated a very marked retardation of yeast growth by this gas, considerably more significant than that produced by alcohol, but fermentations in closed vessels under high pressure show that this is not the case in practice (Section 600). The most important factor in the slackening of fermentation appears to be the accumulation of alcohol. Tait and Fletcher[9] found that retardation occurred in the presence of 0·7% of alcohol and its accompanying CO_2. Reproduction rapidly decreases as the quantity of alcohol rises to 3% by weight, when it becomes very slow or is practically arrested. Fermentation and production of alcohol can, however, proceed until about 8·5% of alcohol is formed and, though multiplication may have become very slow at about 3%, growth of the individual cells continues and their weight may increase by 20%.

Temperature and rousing are the most important controllable factors determining the rate of fermentation with any race of yeast. The rate varies with different races, although the ultimate attenuation they produce under favourable conditions may be the same. It also varies with the physiological state of the yeast, its age, the manner in which it has been stored and, apparently, with its previous history. The last may, however, in many cases be due to accumulation of one type during previous fermentations. It is almost proportional to the pitching rate, within the limits used in brewing, if the yeast is kept suspended and prevented from flocculating in the wort. The concentration of fermentable sugars influences the rate of fermentation, but not within the limits in which they exist in worts until the supply is nearly exhausted. Glucose, fructose, mannose, sucrose and maltose ferment at practically the same rate, within concentration limits of 1–10% (Hopkins and Roberts [10]). Slator found that the generation time, that is the time in which one cell produces two or in which these two multiply to four, etc., during the logarithmic period of unrestricted growth, was 4·1 hours under anaerobic conditions or 2·9 hours in presence of air, under the conditions of his experiments at 68° F.

(561) Cooling and Clarification.

The last stage of fermentation processes comprises cooling and clarification of the wort. It may occupy about half the time in the fermenting vessel, but very little fall in the specific gravity or p_H value of the wort occurs, when a strongly flocculating yeast is employed. With a powder yeast, the specific gravity may continue to fall slowly to the end and the beer may rack with a considerable amount of yeast in suspension. The racking gravity and p_H value of the beer are important factors in its behaviour during storage and stability. The former should be considered in relation with the limiting attenuation of the wort (Section 413), and adjusted to leave the quantity of fermentable matter in the beer that experience shows to be adequate for the condition required after storage in cask or tank. The attenuation at racking is often expressed as a fraction of the original gravity of the wort. Thus a beer may be said to have attenuated to a third, a quarter or a fifth, according as its apparent attenuation is 66·6%, 75% or 80%. An apparent attenuation of 75% or to a quarter gravity represents average practice with a limiting attenuation of about 78 to 82%. Beers with a strongly flocculating yeast are frequently attenuated less, to between one-third and one-quarter, while beers with which greater stability is required are commonly attenuated to one-fifth. Lager beers are generally attenuated rather less than top fermentation ales.

A difference of 5% between the racking attenuation and apparent limiting attenuation means 3° of specific gravity with an original gravity of 1060, and 2·6° with a beer of 1048 original gravity. This is usually sufficient to allow for a persistent condition in cask. A difference of 10% or over 5° of gravity indicates the existence in the beer at racking of an excess of fermentable sugar and conditions that are liable to result in primary yeast fret, with violent condition and fining difficulty, and ultimate acidity through the development of *Lactobacillus pastorianus* (*Saccharobacillus pastorianus*). Lower percentages of fermentable matter lead to lack of cask condition and, in the absence of vigorous primary yeast, to secondary yeast frets. The lack of fermentable matter may be compensated by addition of primings but, if these are rapidly fermentable by the primary yeast present, poor condition may soon supervene and development of wild yeast follow. These points indicate the importance of the control of racking gravity and of the quantity of yeast present in the cask (Section 620). If the limiting attenuation of the wort is not used as the basis of gravity control, the fall of gravity of unprimed samples during 7 or 10 days' forcing provides useful indications.

(562) Rousing.

Active fermentation depends in such large measure on suspended yeast that worts are roused at some stage in every system of fermentation. The extent of rousing and the methods employed depend on the flocculating properties of the yeast and the object to be attained. The pitching yeast is first well mixed with the wort. Subsequent rousing may be directed to stir up sedimentary yeast, "bottom rousing," or to beat in heads already formed, "top rousing." The latter may be carried out by fountain, spray, compressed air or pump type rousers, while buckets are still sometimes employed. These can all bring up wort and return it over the heads, without disturbing the sedimentary yeast to any great extent. The object is generally to stimulate fermentation and attenuation by retarding the rise or settlement of the yeast, by returning heads already formed into the wort and by keeping the yeast in suspension and motion. Bottom rousing, which can also be carried out by compressed air injection or by stirring up the sediment with paddles, is used in bottom fermentations to stimulate fermentation and, occasionally, in top fermentations to help bring the yeast up into the heads and reduce attenuation. The effects thus depend on whether it causes the yeast to remain in suspension or assists its rising to the head.

Fermentations proceed normally in some cases with no other rousing than is effected when the wort is collected or transferred from one vessel to another. The yeast in these cases is of the powder type. In the Union system there is a circulation of beer from the barm backs to the casks (Section 585), and no other rousing is required. Skimming systems are carried out with yeasts that vary greatly in flocculating properties. The amount of rousing is governed by the latter. Frequent rousing is essential with strongly flocculating yeasts and the period over which it is continued is decisive for the attenuation reached at racking. In the Yorkshire system the worts are pumped for a period of half an hour every two or three hours from 24 to 30 hours after pitching until a few hours before the yeast is removed. As soon as the periodical rousing is stopped with yeasts of this type, in whatever kind of fermenting vessel they are used, the yeast separates and attenuation comes practically to an end. If this is too early, the beer will rack with too much fermentable matter and be liable to develop excessive cask condition. Further rousing to postpone skimming is required to prevent this and allow the beer to attenuate rather more.

Every method of rousing is liable to introduce the danger of wort and beer infection. Pumps, main and any other appliance used must be scrupulously clean and should be sterilised. Compressed air should be passed through a cotton wool or other sterilising filter.

(563) Aeration and Yeast Growth.

The effects ascribed to keeping the yeast in suspension were formerly attributed mainly to the activating influence of oxygen, assisted by the removal of CO_2. In practice, an adequate supply of oxygen, represented by the small quantity required to saturate the wort, is necessary to shorten the lag phase and ensure normal attenuation. The wort should absorb this during cooling (Section 506), but rousing or injection of air at the commencement of fermentation is helpful when aeration was inadequate. It is difficult to say whether this is due to stimulation of yeast growth and respiration by oxygen, to its influence on the oxidation-reduction potential of the wort or mainly to stirring up the yeast. Brewery trials have indicated that injection of " Brew-air " is more effective in increasing attenuation than rousing with air. " Brew-air " is the name given to the residue obtained after production of liquid oxygen from air. It is air from which a considerable proportion of the oxygen has been removed, sterilised by the heat developed in compression and supplied in cylinders. At later stages aeration is of no advantage and may be detrimental. It is also very doubtful whether the removal of CO_2, by transferring wort from one vessel to another, has any share in the stimulation of fermentation that occurs.

Pasteur's view that fermentation was a consequence of life without air may be accepted as a general principle. Yeast will grow in absence of air, but growth is greatly stimulated by oxygen and adequate growth is essential in brewery fermentations, which would be very slow if they depended on the increase that occurs during anaerobic fermentation. Yeast demands energy for synthesis of cell material and growth, for which the aerobic breakdown of sugar, associated with respiration, provides a much more abundant supply than the anaerobic breakdown in fermentation. Considerations of this kind support the view that a restricted quantity of oxygen is essential to supply the energy required at the commencement of fermentation, even if it is unnecessary in the later stages. On the other hand, the activities of certain micro-organisms have been found to be influenced by the oxidation-reduction potential of the medium, rather than by the quantity of gas present in the gaseous phase (Section 696). This may apply to brewery fermentations, which may be stimulated by the high degree of oxidation of wort constituents resulting from aeration, rather than by the direct effect of oxygen. It has been found that the r_H value of wort falls during fermentation from 20 or higher to about 9 and that yeast growth is very slow at r_H values below about 12.

The extent to which oxygen acts as a direct stimulant of brewery fermentations thus remains uncertain. Its effect on the metabolic activity of the individual cells has also been a subject of dispute. F. Windisch [11] obtained evidence that the respiratory and fermentative functions of yeast were activated or depressed in the same way by the presence or absence of oxygen and by changes in temperature, supporting the view that they are the result of the same metabolic processes, functioning under different conditions. Investigations were made with yeasts that had previously been grown under anaerobic, aerobic or semi-aerobic conditions, the last being similar to those obtaining in the brewery, and it was found that their fermentative and respiratory activities were both increased or decreased by the conditions of aeration or temperature that operated during the previous fermentation. As a result, Windisch formulated the new biological conception that abundant aeration weakens the metabolic functions of yeast. The importance of oxygen for the energy capacity of the individual cells must be regarded as purely vegetative. It is a powerful stimulant of multiplication and, combined with intensification of energy expenditure, results in a weakening of the mother cells and forced development of the daughter cells. The total fermentative effect of an aerobic fermentation may be greater than that of an anaerobic, on account of the increased number of cells, but the individual aerobically cultivated cells ferment less strongly than the individual anaerobic cells. This applies to cells that are accustomed to powerful aeration, but as soon as yeast has become acclimatised to an anaerobic medium, there is no impairment of the fermentative effect, although the outcrop diminishes. Reference is made in Section 600 to certain conclusions derived by the same author from trials in bottom fermentation breweries, bearing on the influence of oxygen and CO_2.

(564) Change in the p_H value of Wort during Fermentation.

The rapid fall in the p_H value of wort during the earlier stages of fermentation, shown in Fig. 150, and attainment of a value at racking about 1 unit lower than that of the original wort are very significant. They indicate production of conditions which, while favourable to yeast growth, are inimical to the development of many bacteria and increase the stability of the beer. The fall in the early stages of fermentation is probably due to assimilation of buffering substances by the yeast, but there is an actual production of acid substances as fermentation proceeds. Hagues'[12] measurements of the reproduction constant of a Yorkshire yeast during the early stages of growth in a brewery wort indicated two optima, one at p_H 4 and the other at p_H 8–8·5, corresponding with the optimum p_H values for proteinase and peptidases. He found that yeast reproduction was most rapid at the lowest p_H values encountered in brewery worts. This is in agreement with the view that reproduction depends primarily on the assimilable nitrogen of the wort. *S. ellipsoideus* behaved in a manner almost identical with *S. cerevisiae* but *S. pastorianus* and *S. validus* appeared to have a third reproduction optimum, corresponding with an enzyme whose activity was optimum at p_H 6·5. When *S. cerevisiae* and *S. pastorianus* were grown together, the presence of the one was deleterious to the reproductive power of the other.

Parsons [13] suggested that there was a safety p_H value at racking, which varied with different beers and trade conditions, but assured adequate stability. It is usually between p_H 4·15 and 3·85 with top fermentation beers. Higher p_H values or greater alkalinity are favourable to the development of bacteria, particularly *L. pastorianus*. The wort and beer p_H values are usually about 0·4 to 0·5 higher with all malt lager beers than with ales, but greater stability is secured by reducing these. The p_H value of bottom fermentation beers in which unmalted cereals are used generally approaches those of top fermentation beers.

(565) Effect of Sediment on Fermentation.

The coarse sediment that settles in fermenting vessels after good flocculation during cooling appears to influence attenuations in similar manner to a rough surface in the vessels, by activating the evolution of CO_2 (Bishop[14]). The CO_2 is not given off in the gaseous form from yeast, but apparently passes through the cell walls in solution and can produce a supersaturation in open vessels, particularly in the lower strata of the wort. Particles of various substances, of which the coarse sediment is an example, can act as foci from which a rapid stream of gas bubbles is given off in top fermentations and a slower stream in the less vigorous bottom fermentations. These bubbles adsorb protein matter, hop resins and yeast cells on their surfaces and carry them to the wort surface in top fermentations but, owing to their less active evolution, only stir up bottom yeast and cause it to remain in suspension. This accounts for the marked stimulation of bottom fermentations by wort sediments noticed by Windisch. It is common practice to transfer bottom fermentation wort after 24 hours in a collecting vessel, with the object of separating it from sediment. This is generally held to improve the final quality of the yeast crop by removing dead and feeble cells, but F. Windisch [15] found that the yeast left behind was more vigorous than that which settled later. In top fermentations the apparent paradox of a more vigorous fermentation with reduced attenuation results from the rapid outcrop of yeast. Comparatively few bubbles are formed in clear worts, a greater proportion of the yeast remains in suspension, so that fuller fermentation results and there is more sedimentary yeast at racking.

The fine sediment appears to have no influence on the rise of yeast, but Bishop and Whitley found that it did check attenuation, though its effect was less than that of the coarse flocculum. In the case of low gravity worts, in which fermentation is not very active, coarse sediment tends to increase attenuation in a manner analogous to its action in bottom fermentations. Its effects in stronger worts may be summarised as follows,

(1) Increased outcrops of yeast.
(2) Reduced attenuation and higher racking gravities.
(3) Reduction of the quantity of yeast in suspension.
(4) Reduction of the quantity of sedimentary yeast.

The accumulation of yeast head through activation of bubble formation provides an interesting example of the adsorption on the surface of bubbles of proteins or other substances that reduce surface tension. Bishop observed that each bubble rising from a particle of sediment carried up a streamer of yeast cells, which gradually fell, but was driven up again by succeeding bubbles, thus keeping more yeast in suspension and activating fermentation. The opposite effect of reducing fermentation is due to the fact that each bubble rising to the surface is covered with a layer of proteins and yeast cells. Since the gas pressure in small cells is greater than that in larger ones, each small bubble passes its CO_2 into larger ones with which it comes in contact and deposits its burden of yeast and protein on the latter. The result is production of large bubbles armoured with a thick skin of yeast, floating as yeast head on the surface of the wort. If the head is broken up by stirring, the gas escapes and the yeast sinks.

When the bubbles collapse, they leave the adsorbed protein matter with the yeast, which thus becomes enmeshed in a fine network of protein and other matter, giving a very stable head.

(566) Dressing Fermentations.

The very old practice of " dressing " fermentations provides a practical example of the influence of coarse sedimentary matter on the behaviour of yeast. It usually consists in adding malt flour or a mixture of the latter with salt, 5 lb. of sieved malt or baking flour and 1 lb. of salt per 80 barrels of wort, for example. It is used for two purposes, either to stimulate attenuation, or to bring the yeast out of wort from which it does not rise completely, and so prevent the dragging fermentation that may otherwise result during the last few days in the fermenting vessel. Stimulation of attenuation was generally attributed to diastatic conversion of dextrins, but the effect is probably entirely mechanical and can be equally well obtained by means of powdered kieselguhr, pumice or bentonite. Powdered yeast food preparations or calcium phosphate can also be used and may, in some cases, stimulate the attenuating power of the yeast as well as cause it to separate. Any additions of this kind should be sterilised before use, to avoid bacterial contamination.

If the dressing is added before or after skimming it helps to bring the yeast to the surface and thereby reduces attenuation or stops the drifting attenuation that sometimes occurs after skimming. If the dressing is stirred into the wort with the yeast heads already formed, it causes further fermentation by keeping the yeast, that would otherwise have separated, in suspension in the wort. A similar increase of attenuation throngh keeping the yeast in suspension has been found to follow the addition of a small quantity of " biochips," wood meal impregnated with Mammut, to bottom fermentations. The following fermentation may, however, be impeded unless the yeast is cleansed from adhering powder and adsorbed colloidal matter by washing with a dilute tartaric acid solution (Nius[16]).

(567) The Surface of the Yeast Cells.

The possible adsorption of colloidal wort constituents on the surface of yeast provides some problems of practical importance. Diffusion of sugars, protein degradation products, salts, etc., through the cell wall is a necessary preliminary to yeast nutrition and fermentation. Coating with an impermeable layer of gelatinous material might be expected to hinder this and it is generally believed that a coating of this kind may be deposited on the yeast cells if, for example, the removal of protein-tannins during cooling was inadequate (Section 501). That this provides the true explanation of defective fermentation, under conditions that render adsorption of colloidal materials more than usually likely, is open to doubt. Some observations by Ranken[17] on the effects of artificially produced yeast coatings are interesting in this connection. He found that yeasts treated with calcium oxalate and hop resins formed smaller heads and fermented more rapidly than untreated yeast or yeast carrying phosphate deposits. Protein-tannin coatings, on the other hand, caused a very slow fermentation and very bulky, rocky heads. Yeast treated with calcium oxalate settled on the bottom of the fermenting vessel in a powdery form. Froth formed on the wort surface but practically no head. Hop resins caused the yeast to deposit in a compact, adhesive mass.

These differences are equally well explained by the influence of the coatings on the flocculation of the yeast as by any suggestion of hindrance to diffusion of soluble substances into the cells. The calcium oxalate prevents the cells from cohering, so that they will float longer in the wort and offer increased surface for diffusion. The proteins, at the other extreme, cause the cells to cohere and facilitate the raising of clumps to the surface by CO_2 bubbles and subsequent accumulation in the head.

(568) Defective Fermentations.

By defective fermentation is to be understood any deviation from the course normal to the yeast and system employed. It may be marked by undue prolongation of the lag phase and retardation of the rate of fermentation or rise of temperature, by reduction in yeast multiplication or failure to give the usual outcrop, by bladdery heads and, in extreme cases, boiling fermentation with little or no outcrop, by a slow continuance of fermentation and rise of yeast after skimming, by failure to attenuate to the usual degree or excessive attenuation. Some of these abnormalities may be attributed to variations in wort composition and some to lack of activity or changed behaviour of the yeast. The former may be corrected by changes in materials or mash heats, for example by raising or lowering the mashing temperature according as it is desired to reduce or increase the attenuation or by adjustments necessitated by lack of nitrogenous nutrients or bios. Difficulties of this kind are avoided by regular analysis of the worts.

Abnormalities due directly to the yeast are more serious and bring into consideration the desirability or otherwise of a change of pitching yeast. This should be regarded as a last resource, if a suitable yeast has been found and has given satisfaction over a number of generations, unless infection is proved by microscopical examination. Points brought forward in the last Chapter should help in the search for the reason of defects noted. These may be due to gradual segregation of less attenuative yeast in the skimmings selected for pitching, to excess or lack of coarse sediment in the fermenting vessel, to too low pitching temperature or pitching rate, to the use of higher maximum temperatures than are advisable, even to excessive pitching rates. If the abnormalities cannot be attributed to any external factors, it becomes necessary to envisage a change in the behaviour of the yeast itself, which may have been contributed to by improper treatment after skimming. (Section 588.)

" Boiling " fermentations, in which there is an extremely vigorous evolution of gas bubbles with little or no head formation, have proved difficult to explain. They have been attributed to excessive maltose content of the wort, unhealthy yeast and newly lacquered fermenting vessels, but none of these explanations appears to be adequate. It is very liable to occur in light gravity, very clear worts, deficient in colloidal constituents, and may be due in such cases to inability of the yeast to aggregate into the compact masses necessary for a stable head.

The harmful effect of washing top fermentation yeasts may be explained, in part at least, in a similar way, by removal of something that facilitates the flocculation of yeast cells, so that the normal course of fermentation is altered. In bottom fermentations, on the other hand, colloidal matter adhering to the yeast would increase the tendency to sink, since the gas evolution is insufficient to form a head. As a result the fermentations would lag and phenomena associated with " degeneration " would be noticed, if the yeast is not washed.

(569) Yeast Degeneration.

Abnormal conditions or appearances in brewery fermentations are frequently ascribed to " degeneration " of the yeast. They may be due to actual degeneration, this word being given its usual meaning, if the health of the yeast has deteriorated through improper treatment by excessive temperatures, aeration or lack of adequate nutriment. On the other hand, reduction of attenuation is not as a rule caused by improper treatment, since every care is taken to store yeast properly and to use it at such an interval after separation from the wort as experience has shown to be most satisfactory. " Degeneration " of this kind is much more frequently due to use of increasingly flocculating yeasts after each fermentation. In some cases, excessive aeration has been found to reduce the activity of yeast (Section 563), and in others it may be discovered that the yeast is being gradually poisoned by absorption of copper, in which case the outcrops may be sadly reduced and signs of attack on copper fermenting vessels become evident (Section 546).

Staining with methylene blue solution 1 : 10,000 is frequently used as a test for dead yeast cells, but the test may actually be most misleading. Fink and Weinfurtner[18] showed that the p_H value of the liquid in which the yeast was suspended had a material influence on the staining, which greatly increased in more alkaline solutions up to p_H 8. Acidity is not, however, the only factor that influences the methylene blue test. If the yeast is suspended in very pure distilled water, it will stain much more readily than in tap water. 90% of a bottom yeast suspension in a solution of pure sucrose in conductivity water, took up the stain, while only 5% stained in a sugar solution in tap water. Fuchs[19] found that the test was best carried out with washed yeast and recommended that enough of the dye solution should be added to render the suspension blue, not greenish blue, and that only those cells that take a deep stain at once should be considered as dead.

(570) Yeast Changes.

Yeast changes in British breweries almost invariably mean supplies from other concerns, either directly or indirectly. They should be avoided so long as fermentations go on satisfactorily and the quality of the beer is good. There are quite a number of breweries in which no change has been made within living memory, but this ideal can apparently only be attained in favourable circumstances. A change is more usually required every few months or years on account of deterioration in the purity of the yeast, because it fails to give the desired degree of attenuation, despite all changes in materials or mashing procedure or because it ceases to give the necessary outcrop.

Since most yeasts are mixtures of fast and slow or non-flocculating and flocculating types and these tend to be separated into the portions of the outcrop that are used for pitching or sale, a gradual change in behaviour, generally loss of attenuating power, is to be expected. Those breweries that have maintained a strain of yeast for many years may be supposed to have developed a more constant type, either flocculating or non-flocculating with little admixture of the other. It is consequently necessary in making a change of yeast to take particular care to select a type appropriate to the methods of fermentation employed, and obtain it from a brewery where this type has become dominant through long usage. Should a flocculating yeast, for example, be used without adequate rousing, inadequate attenuation will inevitably result. If, on the other hand, a fast yeast is selected and early heads are taken for pitching, a gradual decline of attenuating power will also follow. A change of yeast obtained from another brewery may behave in an anomalous way for one or two fermentations before it works in the manner characteristic of the brewery taking the change. This is frequently ascribed to a change in the composition of the wort, and the time required for the yeast to become acclimatised. Deterioration inevitably occurs if the wort is deficient in essential nutrients and bios. (Chapter XXXVIII). The best pitching yeasts are obtained from beers of 1045–1060 specific gravity. Those from higher gravity beers are liable to be sluggish and constant use in lighter gravity beers tends to rapid deterioration.

No disturbance in the normal course of fermentation need occur when a constant supply of pure yeast is available. This is one of the most important advantages of a pure culture plant, from which a change can be made smoothly as frequently as may be necessary. Every 9 generations is common practice in lager breweries. Purification by the method described in Section 576 may avoid the need for a change on account of bacterial contamination.

PURE YEAST

(571) Single Cell Cultures.

The announcement by Hansen in 1883 that brewery yeast might be a mixture of several races with different properties marked the opening of a new era in brewing, and his suggestion that pure cultures, derived from one single cell, offered possibilities of regularity that were otherwise impossible was soon put to the test at the Old Carlsberg Brewery in Copenhagen. The preliminary results were so encouraging that a continuously working apparatus, designed by Hansen and Kühle for preparing the pure yeast in large quantity, was being used in the brewery by the beginning of 1886. By the end of the year, an apparatus on similar lines but differing in detail, designed by Elion, was in operation at Heineken's Brewery, Rotterdam. Since that time, the use of pure cultures has become almost general in bottom fermentation breweries. Some have their own installations, but others obtain continuous supplies from Scientific Stations or other breweries.

Since each culture is raised from a single cell, selected from what was probably a mixture of races with diverse properties, it is necessary that the characteristics of each should be carefully determined by laboratory experiments and observation in the brewery, in order that the one most suitable for any particular brewery or type of beer may be selected. It is necessary to find which gives the best flavour, adequate attenuation with cask or storage tank condition, brilliance or ease of fining and good crop. This is an arduous task, as it is desirable to grow up sufficient of each to pitch at least ten barrels and some of the selections are likely to prove useless. Experience, gained during the last half-century, in the use of pure yeast has familiarised brewers with the characteristics of numerous strains of bottom fermentation yeast. Several Scientific Stations have a range of cultures, some for particular breweries and others with special properties, from which the most suitable for any circumstances can be constantly obtained. Some of these are characterised by highly attenuative properties, others attenuate the beer to a lower degree. The former settle slowly from the beer, the latter flocculate rapidly. They would, in general, be suitable for stock or quick sale beers respectively. Some may produce special flavours, while others would be suited to worts of high or low p_H value. Most breweries make use of a single strain, but others have two or three for different beers or prefer to mix yeasts of different character.

(572) Preparation of Pure Cultures.

The original sample of yeast from which pure cultures are to be made is taken from a fermenting vessel showing particularly fine characteristics, at the time of most active reproduction when the yeast is in its purest condition. It should be taken 50 to 75 hours after pitching, from top fermentations. Several methods are available for isolating single cells from the sample. If a Dickinson[20] Isolator is available, selected cells can be picked out from a preparation, while actually under microscopic examination. The requirements for this are a microscope with a good mechanical stage, to some fixed part of which the special mechanical attachment designed by S. Dickinson can be clamped. The apparatus has something the appearance of a second mechanical stage and carries a very fine glass needle turned up at the point and of such a length that, by manipulation of the attached movements and of the mechanical stage, the point may be made to traverse every part of the underside of a coverslip on which a mount of the yeast is made on agar. Any single cell can be selected and picked out by the needle to serve as the starting point for the culture.

The following is typical of methods used in lager breweries. The single cells may be isolated from a thin preparation of yeast on the underside of a large coverslip, marked out with numbered squares, and mounted on a moist chamber. The preparations are examined under the microscope, with a magnification of about 100, and the positions of several well-isolated cells recorded in relation with the numbers etched on the glass. After incubation for 24 hours at 68° F. the cells will have commenced to form colonies which, after another 24 hours, will be visible to the naked eye. Any that are sufficiently isolated from their nearest neighbours and are known from the preliminary examination to have developed from single cells can be picked out with a needle and transferred to 50 ml. Freudenreich flasks or test-tubes, containing 25 ml. of sterile hopped wort of about 1040–1045 sp. gr. About a dozen cultures can be made in this way and incubated for 24 hours at 82° F. Three or four 250 ml. Pasteur flasks can be inoculated from each of these and each of the second cultures will serve to inoculate three or four 2-litre flasks, from which the cultures can be transferred to 10- or 20-litre copper vessels, constructed on the same principle as Pasteur flasks. The large vessels should yield 250–300 grams of thick yeast. So far the cultures are made in the laboratory. A photograph of a brewery laboratory with some of the necessary culture vessels is shown in Fig. 151.

(573) Pure Yeast Culture in the Brewery.

Several types of apparatus are available for growing up the laboratory cultures until sufficient yeast is obtained for pitching in the brewery. These consist essentially of a wort steriliser and one or more propagating vessels and are generally variations of the apparatus first used by Hansen at the Carlsberg brewery in 1883. The pure yeast room of the Tuborg Brewery, fitted with Hansen vessels, is shown in Fig. 152. The propagation cylinders on the right of the photograph are made of tinned copper but stainless steel is to be preferred. Each has a capacity of 150 litres (33 gallons) and is jacketed. They are sterilised by blowing steam through the whole apparatus for several hours, after which 100 litres (22 gallons) of boiling hopped wort of 1045 gravity, previously sterilised in a closed cylinder in another room, is run in. Sterile air is immediately and continuously blown over the surface of the wort under a low pressure and the wort cooled to 7° C. (44·6° F.) by passing cold water through the jacket. The wort is inoculated by blowing 1 litre of the pure culture from a container filled in the laboratory and connected by rubber tubing to the small side tube of the cylinder. Fermentation is completed and the yeast settled in ten days, the temperature rising slightly above that of the room, which is maintained at 7° C. The bulk of the beer is run off, leaving 25 or 30 litres with the yeast. This is stirred by means of a propeller operated by a handle at the top of the cylinder and run out into a sterilised bucket. Further generations of pure yeast can be grown up in the cylinder by running in 100 litres of sterile wort. It is possible in this way to get 100–200 generations of pure healthy yeast from one original culture, the gravity being regulated each time a growth is taken from the cylinder.

The yeast collected in the bucket is thereby exposed to the air and can no longer be considered as necessarily pure. It is transferred to 10 Hl. (6 barrels) of wort in one of the open glass-enamelled vessels, shown to the left of the photograph, where fermentation goes on for another 10 days. 25 to 30 litres of thick yeast is obtained from each of the vessels and transferred to a brewery fermenting vessel of 57 Hl. (35 barrels) and used for 8 or 9 generations in the brewery, after which it is discarded and replaced by fresh cultures.

A continuously acting, pure yeast culture apparatus designed and patented by the Schwarz Laboratories, New York, is shown in Fig. 153. It consists of stainless steel sterilising and propagating vessels each of about 4 barrels capacity, and should be installed in a special room maintained at 50° F. for bottom yeast or at about 58° F. for top yeast. The lower part of the steriliser is jacketed for heating or cooling. The bottom of both vessels are dished to permit complete drainage and easy cleaning. All pipes, cocks and gauges are also constructed in a manner to facilitate cleaning and sterilisation. The air inlets are provided with cotton wool filters, and the CO_2 outlets with water seals. The apparatus is tested for air leaks under a pressure of 15 to 20 lb. previous to sterilisation, which is effected by boiling water in the two cylinders by means of direct steam and jacket heating. The liberated steam sterilises the pipe lines and valves. The cotton wool filters are separately sterilised for two hours at 298° F. Four barrels of bright wort from the hop back are introduced into the steriliser, while it is cooling and still under pressure of sterile air. This is boiled for half an hour by steam in the jacket and then aerated by injection of sterile air for half an hour. The wort is then cooled by water in the jacket to 50° F. for bottom yeast, or 58° F. for top yeast, and allowed to settle for about an hour.

Twelve gallons of the sterilised wort are forced by sterile air into the propagator. About 300 grams of the pure yeast are introduced under sterile conditions and roused in the wort with sterile air. A further 18 gallons of wort are transferred to the propagator the next day, with 44 and 70 gallons the following two days to complete the 4 barrels. The wort is fermented completely under a pressure of 1 or 2 pounds, and the finished beer forced by sterile air to a storage tank. Four barrels of sterile wort, which has meanwhile been prepared in the steriliser, is transferred to the propagator. The next day this wort is fermenting vigorously, and is transferred to a 65-barrel fermenting vessel specially set aside to provide pitching yeast for the brewery. Sufficient yeast is left behind to start another 20 gallons of wort, so that the apparatus works continuously, providing fresh yeast for the brewery vessel, every 10 days with bottom yeast and every 7 days with top yeast. The cycle can be continued for two years, when it is advisable to commence again with a fresh culture. The 65-barrel brewery vessel is erected in a special room, preferably supplied with pure air, and should be thoroughly cleaned before use; 20 barrels of cooled wort are added to 5 barrels from the propagator, and a further 40 barrels on the second day. Sufficient yeast should be obtainable from this to pitch about 350 barrels of wort.

(574) Pure Yeast for Top Fermentation Beers.

In the very early days of pure yeast culture, there was considerable debate as to whether a top fermentation yeast would satisfactorily carry through the secondary cask fermentation, which was then almost universal with the better types of beers. Brown[21] summarised the results of an extended trial of a number of pure races of yeast carried out at Worthington's Brewery, Burton-on-Trent, from 1885 onwards, to the effect that "after due consideration of the results, obtained during eight or nine years of such work, there is no doubt left in my mind that the pure yeast beers, on an average, did not show any marked superiority over those brewed with the ordinary yeast as regards flavour, brilliancy and general qualities. In fact, in one respect, the advantage was with the last mentioned, since the beers from the pure yeast, unless stimulated by the addition of a little diastatic malt extract at the time of racking, were slower in conditioning than the corresponding samples from the ordinary yeast."

Results of a similar kind were obtained in some other breweries with the consequence that, despite success recorded at a few breweries, pure yeast has not been adopted in the British Isles for top fermentation beers, though it has been to some extent elsewhere. To overcome the difficulty with secondary fermentation, Van Laer[22] contended that a mixture of selected races was

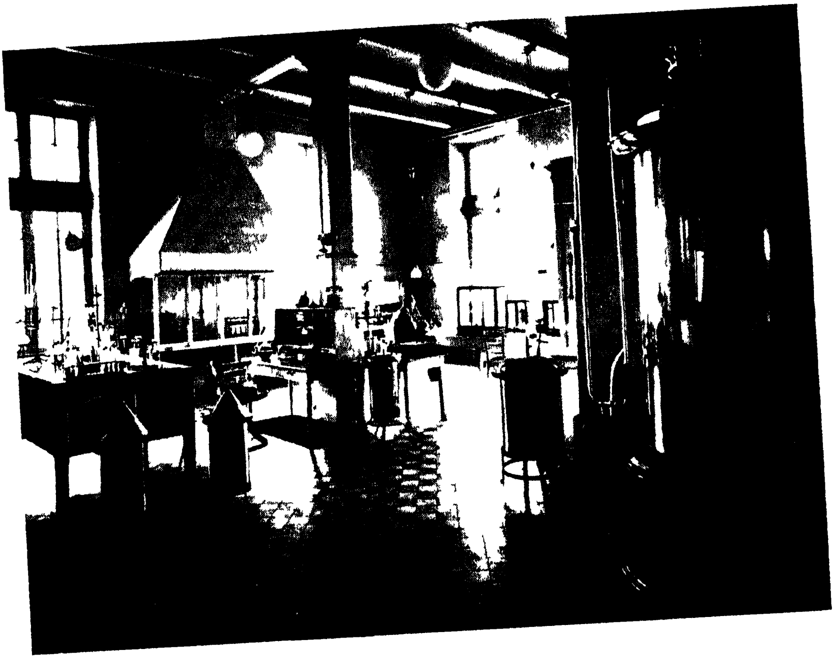

FIG. 151

BREWERY LABORATORY (BRASSERIES ET CHAMPIGNEULLES, FRANCE)

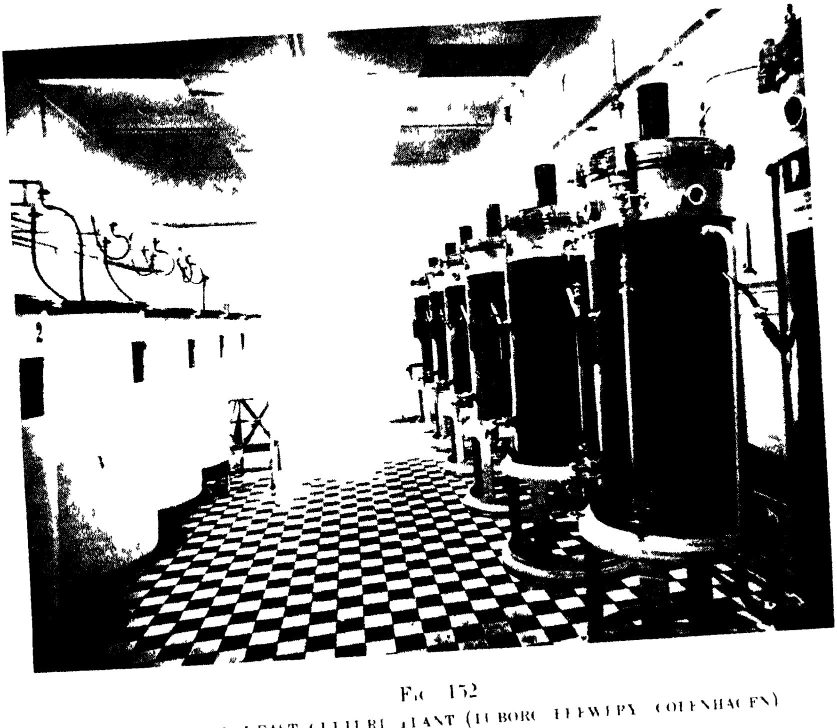

FIG. 152

HANSEN PURE YEAST CULTURE PLANT (TUBORG BREWERY, COPENHAGEN)

PURE YEAST CULTURE

Facing page 800

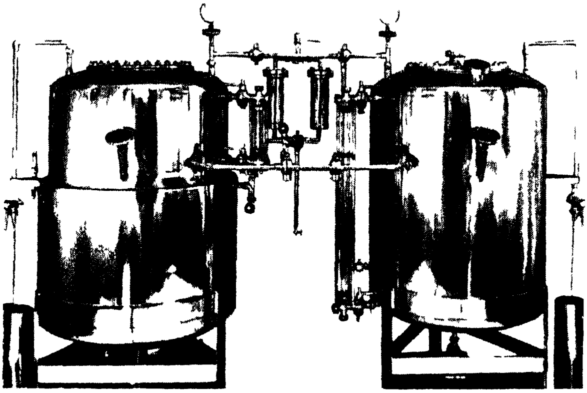

FIG. 153

SCHWARZ TYPE YEAST CULTURE PLANT

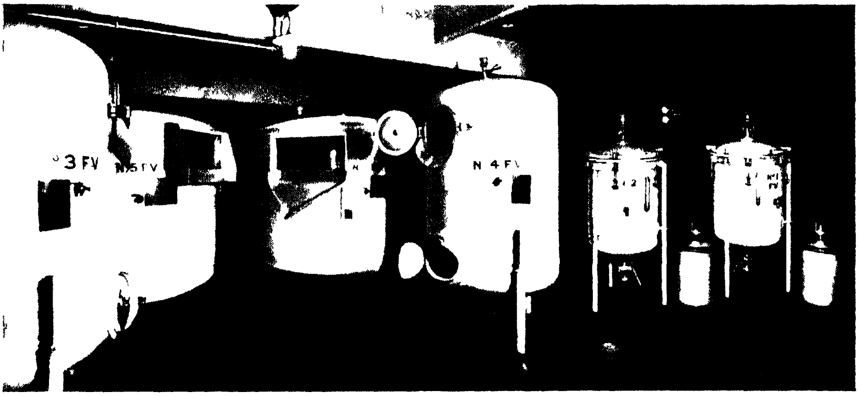

FIG. 154

TOP FERMENTATION YEAST CULTURE PLANT
(WHITBREAD AND CO., LTD., LONDON)

PURE YEAST CULTURE

Facing page 801]

essential, a procedure that was strongly contested by Jörgensen,[23] who constantly urged that, by methodical selection of a suitable race, an element of certainty was introduced into the fermenting conditions that was impossible when a mixed yeast of unknown composition was employed. Confirmation of this was obtained by Miller and Hyde[24] by two years' trial in a Manchester brewery, where the conclusion was reached that the position taken up by Van Laer was untenable, that pure yeasts of single race do exist which are able to induce both primary and secondary fermentation, that the resulting beer is superior to and more uniform than a beer brewed with a composite yeast, and that no diastatic agent is required to bring about satisfactory secondary fermentation.

Pure yeast does not, however, appear to be so essential in top fermentation breweries as it has proved to be to maintain regularity of fermentation in lager breweries. A more or less satisfactory natural selection of races is achieved by elimination of the first and last outcrops and use of the middle yeast only for pitching. The yeasts used probably invariably contain at least two races with different characteristics, but the quantity of wild yeasts in a healthy pitching yeast is not nearly so serious as might be expected. Bottom fermentation temperatures, on the other hand, are so low as to lengthen the lag phase before the yeast commences its active growth and other micro-organisms have a better opportunity to develop. In many top fermentation breweries, the same yeast is maintained in use for many years without any of those signs of degeneration or infection that set in much more rapidly with bottom yeast and for which no method of replacement can give such consistent results as a succession of identical cultures at sufficiently short intervals to eliminate the chances of degeneration.

The so-called secondary fermentation of top fermentation beers in casks is, under normal conditions, carried through by primary yeast. The true maturation accompanied by development of special flavours, requiring several weeks or months in cask, is no doubt frequently the result of the activity of other organisms. The nature of these organisms and the character of the beer produced depends on infection from the plant or on the presence of a small quantity of the organism in the pitching yeast and is not under control. As a result the effects may be extremely variable, although in many breweries they are usually very constant. No serious advance has been made in bringing this type of maturation under control by introducing selected cultures of the necessary organisms after primary fermentation is completed.

An argument against the employment of pure yeast, with the trouble involved in its production, is that infection from the yeast itself is comparatively rare and that it almost always comes from the plant and negatives any advantage that would be gained by the use of pure yeast. This argument assumes that infection is unavoidable in breweries, as it certainly is with some old types of plant, but it is rapidly losing its force with modern improvements. In many cases the ordinary yeasts are used for years without any necessity for a change or any apparent alteration in their properties and with only sporadic outbreaks of wild yeast infection. When these do occur the damage may be so serious and the anxiety caused by the necessity to find a suitable change so great that a very strong argument is found for the installation of a pure yeast plant from which supplies can be constantly obtained, and the temptation to postpone a change until too late avoided.

The very greatly diminished output of old stock beers and the preference accorded to beers which have undergone nothing more than a short period of conditioning strengthen the argument for renewed consideration of the possibilities of pure culture in top fermentation breweries. There is already a considerable amount of experience in breweries in several parts of the world showing that there is little fear of change in character of the beer resulting from adoption of a selected pure culture in place of an ordinary mixed yeast, and that fermentation and conditioning are more regular if a suitable selection has been made, but in some breweries it has been found advantageous to use together two cultures of different types, as is customary in some bottom fermentation breweries.

(575) Purified Yeast.

A modified system, which avoids the difficulty of finding a single race capable of carrying through the primary and secondary fermentation in the established manner of the brewery, and may incorporate the advantages afforded by the use of two or more selected races together, if such advantages actually exist, has proved a success after extensive trial. This system, described by B. M. Brown,[25] provides a yeast which, if not pure, is purified and probably represents a fair average of the original yeast. It fulfils one of the main objects of pure yeast culture, the production of a regular supply of pitching yeast free from disease organisms and in sufficient quantity to replace the yeast in use before degeneration sets in. In the particular case described the object was to obtain highly attenuative yeast of constant character, to replace the current pitching yeast before the latter had degenerated, which previously had presented a serious difficulty necessitating frequent changes.

In the method adopted a plate culture in wort agar is prepared from a suspension of the yeast of such a dilution that 20 or 30 colonies appear on the plate. 10 or 15 of these are selected microscopically and transferred to a single tube of hopped wort from which, after 24 hours' incubation, a laboratory stock culture may be prepared on an agar slope. A test-tube of hopped wort of 1040 specific gravity is inoculated from the slope and from the test-tube 200 ml. of wort. In practice the latter becomes the starting point for cultures and is kept in active growth by giving it fresh wort twice a week. From this 25 ml. is pitched in 300 ml. of wort, the whole of which after 24 hours' incubation is pitched in 2 litres. The culture is similarly increased through 4½ gallons and 20 barrels up to 40 barrels, from which pitching yeast is obtained in 10 days from the start. Between successive pitchings there are intervals of from 24 to 72 hours, the worts in all cases being aerated by suitable methods; the flasks are shaken, the 4½-gallon and 10-barrel vessels aerated by compressed air or cone rousing. A first wort of 1073 specific gravity is used for the 1-barrel vessel, the others are given hopped wort of the gravity of the beer for which the yeast is intended. Up to 4½ gallons the worts are sterilised by boiling, the larger vessels are filled with ordinary brewery wort.

The plant and process are simpler than in the pure yeast system, but every care must be taken to avoid infection. The larger vessels are of aluminium erected in a specially constructed room kept constantly at 60°–63° Fahr. with pure air. They are filled from vessels in a room above in which brewery worts are collected and declared. The final fermentation is carried to completion, the yeast being allowed to work off over a lip into shallow movable vessels, in which it is drained and kept in a cold store until required. The average crop is 50 lb. calculated as pressed yeast, from each 40-barrel vessel. Fig. 154 is from a photograph of the plant used.

(576) Purification of Pitching Yeast.

As long ago as 1876, Pasteur showed that yeast contaminated with certain bacteria could be purified by growing it in slightly acid solutions of cane sugar. Brown[26] developed from this observation a practical method for purifying pitching yeast by two successive cultures in wort of about 1050 sp. gr. acidified by 0·1% of tartaric acid, followed by a third growth in wort to which no acid is added. Vessels of 2-, 6- and 15-barrel capacity or proportional dimensions are suitable, yeast collected from one being passed on to the next. An addition of 5¾ oz. per barrel of tartaric acid (0·1%) reduces the p_H of the wort to about 3·9, which has no ill effects on the activity of yeast, but is sufficiently unfavourable to the development of the "short thick rods," *F. proteus*, commonly found in yeast, to eliminate or very much reduce the infection. *L. pastorianus* (*Saccharobacillus*) is probably not killed, but greatly reduced in quantity by dilution. The contention that the development of wild yeast is favoured under these conditions appears to be erroneous.

(577) Yeast Analysis.

Microscopic examination of pitching yeast is insufficient to establish the presence of wild yeasts, unless their quantity is abnormal and their shapes markedly different from those of the primary yeast. An approximate measurement of the degree of contamination of bottom fermentation yeasts can be obtained by Hansen's method of yeast analysis by spore culture on gypsum blocks at 25° and 15° C. (77° and 59° F.). At 15° C. wild yeasts are stated to form spores much earlier than most primary yeasts, but at 25° C. some culture yeasts sporulate equally as rapidly as wild yeasts. As the relative proportion of wild yeasts increases towards the end of fermentation and in the upper layers of bottom fermentations when the yeast has settled, a sample is taken in a sterile flask at the close of primary fermentation from the clearer layer and set aside for a few hours to allow the yeast to settle. Portions of the sediment are transferred to gypsum blocks and examined for spores after 40 and 72 hours at the higher and lower temperature, respectively. It is claimed that from 0·5 to 1·0% of wild yeast can be detected by this method.

Jörgensen found that, in some cases, a temperature of 10°–12° C. was to be preferred for top fermentation yeasts, some of which spored readily at the higher temperatures, though these showed up the wild yeasts in most cases. The method is, however, not very satisfactory for top yeasts, since 0·5 to 1·0% of wild yeasts would be considered excessive. Brown found that wild yeasts in the proportion of 1 in 5,000 could be readily detected by fermenting a sterile wort with the yeast to be tested and afterwards forcing the beer produced at 80° F., with and without aeration.

(578) Summary.

The varied fermentative activity of different types of yeast is attributed largely to their flocculating properties. Vigorous fermentation and rapid attenuation only occur when the yeast is suspended in the wort. Flocculation hastens its separation in the heads under the influence of rapid gas evolution in top fermentations, or its sedimentation in the slower bottom fermentations. Fermentative activity is also influenced by the physiological condition of the cells, which may be weakened by age, by high temperature or excessive aeration in previous fermentations and by improper storage, etc. Degeneration of yeast, marked by reduced attenuating power, may be due to these conditions or may be only apparent and caused by segregation of weakly attenuating, flocculating types in the heads or sediments and their subsequent use for pitching. When one type predominates in a mixed yeast, use of the middle heads or sediments for pitching may serve to select and perpetuate this type by eliminating others which differ materially from it. If the pitching yeast consists of types differing more markedly in their flocculating properties, the ordinary method of selecting pitching yeasts tends to segregation of the more strongly flocculating types, with reduction of the attenuating power after each fermentation. The attenuating powers of flocculating and non-flocculating or powder yeasts are actually the same, as can be shown by comparative fermentations in which the wort is continually shaken.

The flocculating property of yeasts largely determines the extent of rousing in the brewery. Very little rousing is required with a powder yeast, since it remains in suspension. With flocculating yeasts, the heads must be continually roused back into the wort. or sedimentary yeast in bottom fermentations stirred up, in order to continue attenuation. Cessation of rousing, under such conditions, leads to inadequate attenuation at racking and unstable beers, since the excess of fermentable sugar permits of excessive fermentation by primary yeast in cask or development of wild yeasts or bacteria, if the beer is racked with too little primary yeast to ensure adequate condition. The influence of aeration on fermentation is not so great as was formerly thought. A certain amount of oxygen is required to assure the reproduction that is essential to a steady fermentation, but this is probably supplied by saturation of the wort at pitching. Rousing in the early stages may provide additional oxygen when that absorbed during cooling was inadequate, but oxygen at a later stage of fermentation is harmful and may conduce to instability, rather than stimulate primary yeast.

During the early stages of fermentation there is a rapid fall in the p_H value of the wort, through assimilation of buffer substances and, later, through production of acidic substances.

This increase of acidity is most important in respect of beer stability, since it assures conditions that are inimical to the development of bacteria but represent the optimum for yeast growth. A p_H value between 4·15 and 3·85 is usually regarded as safe for top fermentation beers. The actual safety point varies with different beers and would be at a lower p_H value if greater infection is expected or storage is lengthy.

Coarse sedimentary matter, by providing foci from which CO_2 is briskly evolved, assists the raising of yeast to the heads and, consequently, reduces attenuation. Dressings of malt flour and salt are frequently made with the same object, to hasten the rise of yeast in dragging fermentations with a non-flocculating type. If, however, the dressing is stirred into the worts with the yeast heads already formed, it assists in keeping the yeast in suspension and so increases attenuation.

It is probable that all British pitching yeasts, not obtained from pure cultures, are mixtures of two or more types, though a single strain would appear to predominate in yeasts which have been maintained without change for very many generations. The different strains existing and their properties are very imperfectly known. In many cases, such signs of "degeneration" as lack of attenuating power, fall in reproduction or dragging fermentation are due to segregation from the mixed yeast and use for pitching of the more flocculating strains, to lack of aeration during refrigeration or selection of a yeast not well adapted to the fermentation system. The unusual behaviour of a yeast is more often due to treatment which is not suitable to the strain imported. Too much or too little rousing may be adopted through imperfect knowledge of the yeast's normal behaviour. A change has always to be considered with caution, and should be taken, when necessary, from a brewery with a well established and known system of fermentation. The uncertainties involved in changes may be avoided by use of a pure yeast plant, and selection of a suitable type. The application of pure yeast to top fermentation has been adversely criticised, but the decline of beers which derive their character from wild yeasts, and their replacement by beers which depend only on the primary yeast for cask condition, have removed the chief objection to its use. A continuous supply of pure yeast of suitable properties provides the surest means for securing regularity in beer character.

Deterioration and early need for another change is, however, certain to occur, whether the yeast be from another brewery or a pure culture, if the wort is lacking in essential nutrients, particularly nitrogen and bios. Variations in microscopic appearance frequently give rise to disquiet. Change of the cell shape from round to oval is widely attributed to lack of nutrients in the wort, resulting in transformation to a form which gives a greater surface to the cell than is provided by a sphere. It may, however, be due in some cases to segregation of a particular race from a mixed yeast in which it occurred in relatively small quantity. A change of this kind is occasionally accompanied by increase of attenuative activity.

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CHAPTER XXXIII

TOP FERMENTATION

FERMENTATION SYSTEMS

(579) Yeasts and Fermentation Systems.

The principles discussed in the last Chapter apply to all brewery fermentations. It remains to be described how they are put in operation in different types of plant and fermentation systems. The latter can only be described in broad outline, as the details of procedure vary in different breweries. Their most distinctive features consist in the methods adopted for keeping the yeast in suspension in the wort and for removing the outcrop. It may be futile to speculate on the origins of flocculating and non-flocculating yeasts, and on the development in different parts of the country of fermentation systems apparently designed to suit one type or the other. It is impossible to say whether the systems have produced the yeasts by separation of the most appropriate type from an originally widely distributed mixed yeast, or whether the yeast indigenous to a certain locality has led to development of the system, and to what extent they are both dependent on the characteristics of the local barley. However the two very distinct types of flocculating and powder yeasts have arisen, it is essential to consider fermentation systems in relation with them. The systems are characterised by varying degrees of rousing. Use of a flocculating yeast in one in which there is little or no rousing inevitably leads to reduced attenuation, while a non-flocculating yeast would be equally inappropriate where much rousing is practised. The systems may be differentiated, according to the manner in which the reproduced yeast is separated from the wort and the type of vessels employed, as

(1) Skimming
(2) Dropping
(3) Burton Union
(4) Yorkshire Stone Square.

(580) Pitching Rates and Temperatures.

By pitching rates and temperatures are meant the weight of yeast added to unit volume of wort and the temperature of the wort when collection in the fermenting vessel is completed. They are practically the same in all systems, but vary to some extent in different breweries and in the same brewery according to the specific gravity of the wort, the activity of the yeast and other circumstances. Pitching rates are generally expressed in pounds of thick liquid barm per barrel. About 20–30% less of pressed yeast is required. Representative figures are given in Table 221. The racking temperature shown there is frequently difficult to attain in the summer unless the liquor is refrigerated. In many breweries the wort is not brought below 58°–60° F., but lower temperatures are to be advised, to ensure delivery in casks at a temperature below that of the retail cellar. They would, however, be of little advantage if the temperature between racking and dispatch is allowed to rise.

TABLE 221.—AVERAGE PITCHING RATES AND TEMPERATURE (POUNDS THICK LIQUID BARM PER BARREL)

Wort, sp. gr.	Pitching rate lb.	Temperature F.		
		Pitching	Maximum	Racking
1030	$\frac{2}{3}$	60°	66°	53°–54°
1040	$\frac{3}{4}$	59°	67°	,,
1050	$1\frac{1}{4}$	58°	68°	,,
1060	$1\frac{1}{2}$–$2\frac{1}{2}$	57°	69°	,,
1080	2–3	58°	70°	,,

The yeast is generally mixed with a small quantity of wort at about 65° before pitching, but may be poured directly into the fermenting vessel and mixed there with the incoming wort at about 65° F. In some cases the flow of wort provides all the mixing required, but, in others, this is accomplished by rousing. If the yeast appears to require invigorating, it may be mixed in a yeast pail with wort at between 65° and 70° F. and allowed to come into active fermentation before mixing in the fermenting vessel. Yeast foods, if used, may also be added at this time. The final temperature of the collected wort, or pitching temperature, is adjusted by gradual reduction of temperature at the refrigerator.

(581) Course of Fermentation.

The progress of fermentation is marked by similar phenomena in all systems, with variations depending on the activity of the yeast and the design of fermenting vessels. The curves for temperature, specific gravity and p_H in Fig. 150 represent what might be called an average fermentation. The fall of specific gravity is steeper with a rapidly attenuating yeast and slower with a flocculating yeast, with which the final specific gravity is usually rather higher. The period of fermentation, cleansing and cooling varies from 5 to 8 days, a wort pitched on a Monday being racked on the following Friday to Tuesday mornings. The average is 6 or 7 days in all systems. Three phases in the formation of the heads in a healthy fermentation are shown in Fig. 155. The froth that covers the wort within the first six hours after collection gradually gives way to small " cauliflower " and then larger " rocky heads," carrying dark masses of resinous and protein matter from the wort. These are skimmed off to avoid redispersal, with consequent rough bitterness in the beer. This is followed by gradual rise of a rather frothy head to a height varying from 2 to 8 feet, according to the depth of wort and vigour of fermentation, until about the 30th to 42nd hour after collection, by which time the maximum temperature, varying from 66°–70° F. according to the specific gravity of the wort, may have been reached. This head then commences to collapse, marking the time for transference to cleansing vessels in some systems. When the wort remains in a single vessel, the frothy head is gradually replaced by the accumulation of reproduced yeast, settling into a compact mass (Fig. 155*b*) as it is ready to be skimmed off. The yeast is then removed as it is formed, until a covering ½–1 inch thick only is left. This frequently takes the characteristic form shown in Fig. 155*c*, as the wort is cooled to the racking temperature and held at its lowest point for settling during the last day or two.

(582) Control of Fermentation.

Fermentations are controlled by periodical determination of the temperature and specific gravity of the wort and inspection of the yeast heads. The declaration is made from the dip and specific gravity of a sample taken immediately after the worts are collected when the pitching temperature is verified. The next sample is taken about 12 hours later, when fermentation should have started. Temperature rises rapidly after this and is read at regular intervals, every 3 hours in some breweries during the first 48 hours. Samples are taken at the same time for specific gravity then, as attenuation slackens, at 6- or 12-hour intervals until it has substantially ceased and, subsequently, each morning until racking. The readings are recorded on a chart or board attached to the fermenting vessel, as shown in Fig. 156, the flow of liquor in the attemperators being adjusted

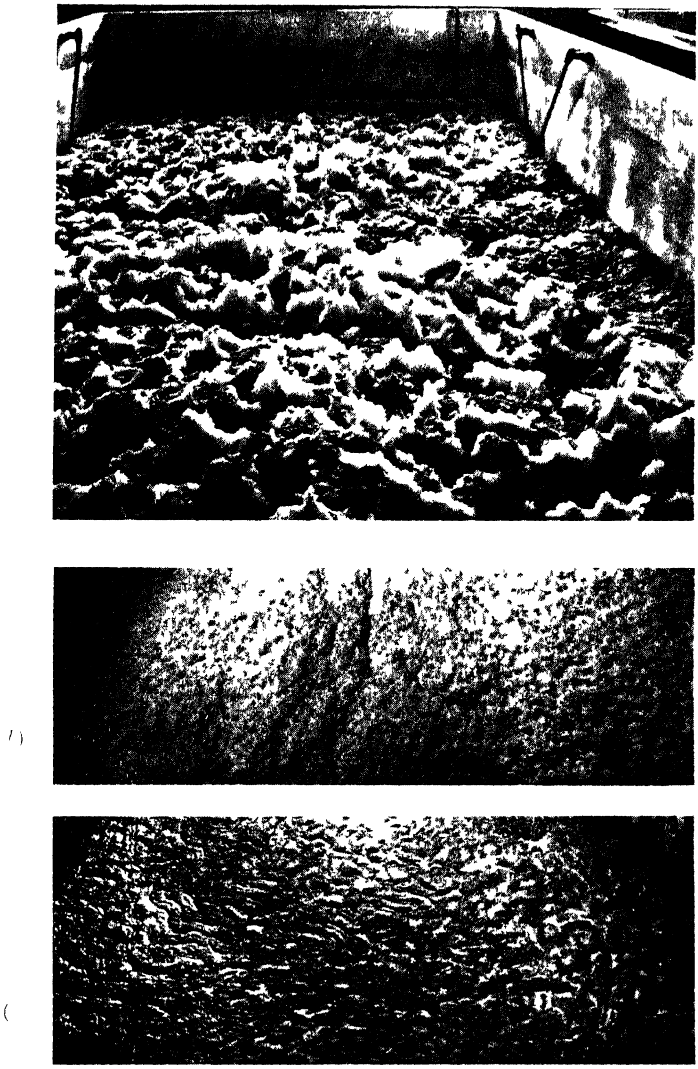

FIG. 155

TOP FERMENTATION YEAST HEADS

(a) EARLY ROCKY HEADS 12-24 HOURS AFTER COLLECTION

(b) YEAST HEAD 54-60 HOURS

(c) YEAST COVER AFTER SKIMMING 4TH-6TH DAY

To face p. 810

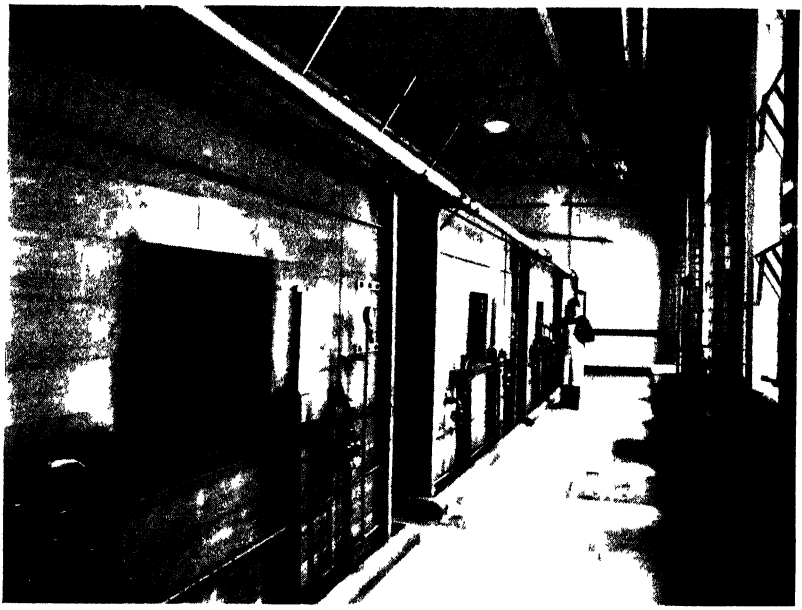

FIG. 156

WOODEN SQUARES CAPABLE OF BEING SEALED FOR CO_2 COLLECTION
(THE BRISTOL BREWERY GEORGES AND CO LTD BRISTOL)

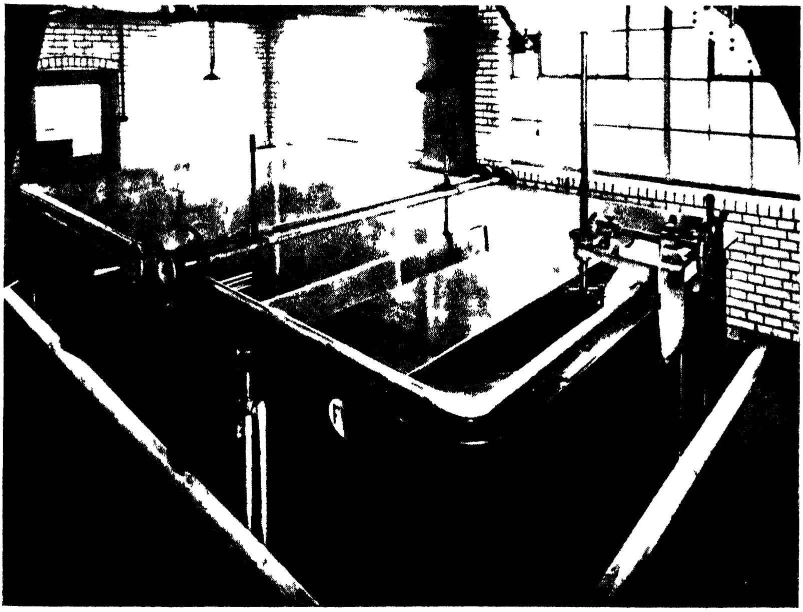

FIG. 157

PARACHUTE AND SKIMMING BOARD (HODGSON'S KINGSTON BREWERY CO LTD KINGSTON)

SKIMMING SYSTEM

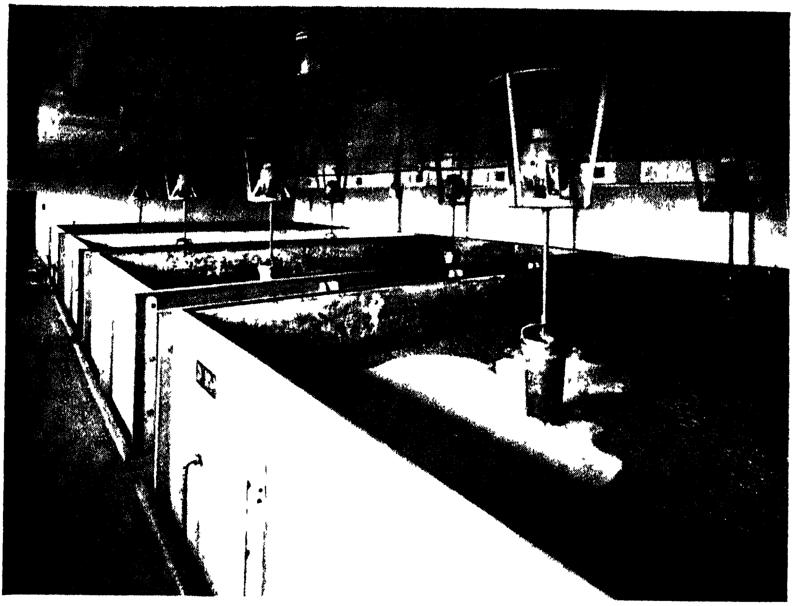

Fig. 158

CONE ROUSING APPARATUS IN WOODEN FERMENTING VESSELS. AIR CONDITIONED ROOM
(FREDK. SMITH LTD., BIRMINGHAM)

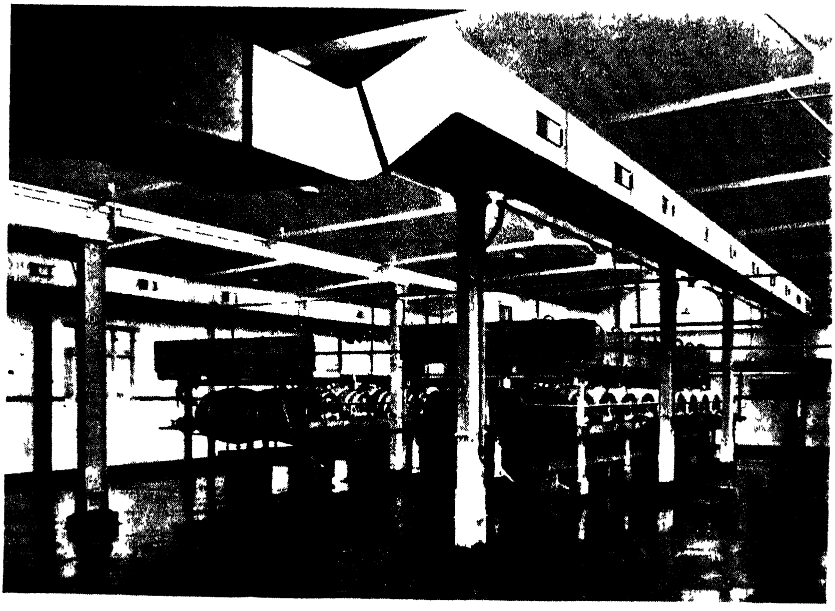

Fig. 159

AIR-CONDITIONED UNION ROOM
(WATNEY, COMBE, REID AND CO. LTD., MORTLAKE)

ADAPTATION OF AIR CONDITIONING TO EXISTING ROOMS

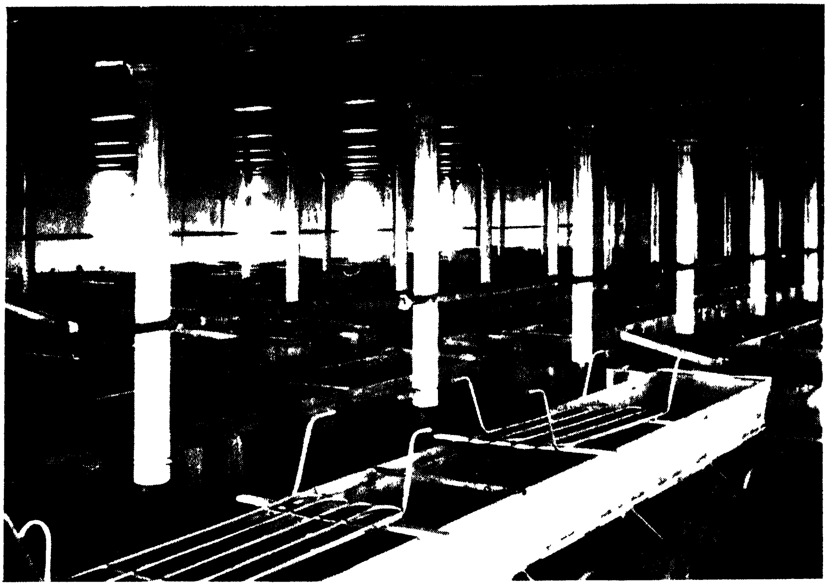

Fig. 160

UNION ROOM (IND. COOPE AND ALLSOPP LTD., BURTON ON TRENT)

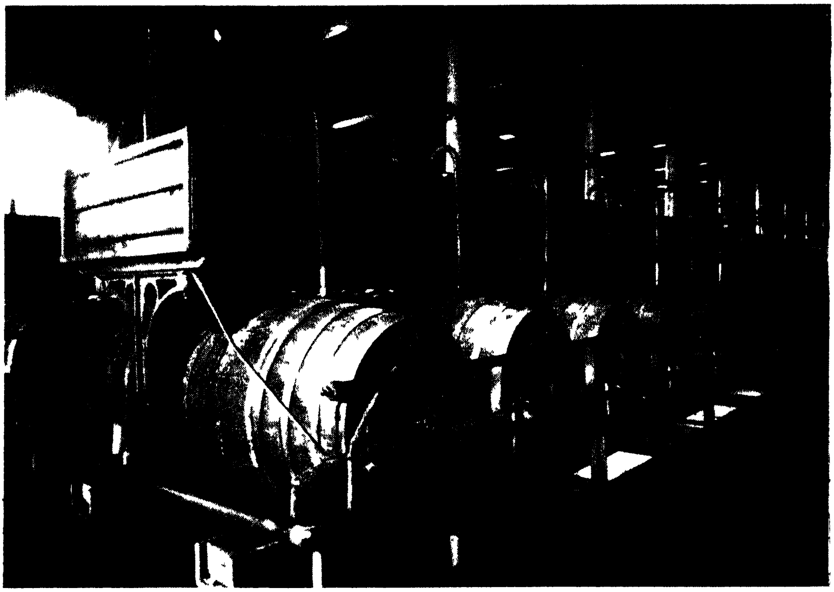

Fig. 161

UNION SET (IND. COOPE AND ALLSOPP LTD., BURTON ON TRENT)

BURTON UNION SYSTEM

Facing page 311

in accordance with the observations. The outlet of the attemperator frequently delivers into an open funnel, so that the flow of liquor can be seen to facilitate regulation. If a leak occurs in the coil, wort is sucked into the attemperator and can be detected by discolouration of the outflowing water. The funnels are sometimes enclosed in sight-glasses.

Temperature control is exceedingly important in all systems. The pitching temperature should be as low as but no lower than is consistent with a steady start of fermentation during the first 12 hours after collection. It is normally rather higher with low gravity worts than with strong, as shown in Table 221. Undue prolongation of the lag phase is evidence that the pitching temperature was too low or that the yeast is lacking in activity, and will give opportunity for any wild yeast present to commence development. Early formation of a head provides protection against aerial infection. The rate of attenuation and rise of temperature varies in different breweries, but may be taken as about 1 degree of specific gravity every 2 hours, with a rise of 1° F. every 8 hours or for every 4 degrees of gravity lost. The temperature should rise steadily and require no checking for the first 36 hours, and sometimes not for 48 hours. It is often necessary to start the attemperator liquor slowly at about these times, or earlier in more rapid fermentations, to prevent the normal maximum temperature being overpassed. The maximum temperature is held during skimming and generally for 6 to 12 hours thereafter, when the flow of attemperating liquor is increased and the temperature of the beer brought down to that required at racking, by about 1° F. every 4 to 6 hours. It is most important that there should be no sudden chilling or check to yeast activity at any point, either by excess of attemperation or a very cold room. A gradual and steady rise and fall of temperature are aimed at in all cases.

(583) Skimming System.

This system is the simplest and most generally adopted. The entire fermentation is carried out in one vessel, the yeast head being skimmed off the surface of the wort and transferred to a yeast back or receiver in some convenient manner. A funnel-shaped "parachute," such as that shown in Fig. 157, is widely used for the purpose. This can be raised or lowered as required, and adjusted just above wort level for skimming. The yeast is drawn to the parachute in the vessel illustrated by means of a metal-covered board which can be raised or lowered and propelled by two handwheels. The Griffin yeast slide is used in many breweries. It consists of a plate, sliding in grooves over an aperture in the side of the vessel. It entirely closes the aperture, but can be lowered by means of a wheel to just above wort level, when the yeast is skimmed over a chute at the top of the slide. Yeast may alternatively be removed by suction, as described in Section 594. Yeast was formerly collected in yeast backs of wood or slate, situated on a floor below the fermenting vessels. These are, however, regarded as dangerous from the point of view of infection. They are, consequently, generally replaced by other methods of collection in newer plants. These may be yeast wagons, such as are shown in Fig. 167, which are conveniently placed under the delivery pipe from the parachute or yeast slide, and serve to convey the yeast to the storage room. Suction is also widely used to transfer the yeast to closed cylinders.

Many vessels have an aperture in one side, cut nearly to wort level, and closed by yeast boards to contain the head. An opening of this kind or a parachute allows CO_2 to flow from the vessel. This point is probably worthy of attention when installing air conditioning in rooms in which vessels with no such provision for escape of CO_2 are used. No draught or strong current of air is permissible, but a gentle circulation which favours removal of excess CO_2 appears to be of advantage.

Fast yeasts of the Burton type and slow, flocculating yeasts are both used in the skimming system. It is essential that the amount of rousing should be adjusted to their particular requirements (Section 562). Its main object is to keep the yeast in suspension, and so enable it to bring the gravity of the wort down to that required at racking. No more rousing should be given than is necessary for this purpose. Little or no advantage is gained from aeration (Section 563), except at the very early stages, when air absorption during cooling was insufficient. The yeast is well mixed with the wort at pitching, and no more rousing may be required with a powder yeast but, if a strongly flocculating yeast is employed, rousing should be as efficient as in the Yorkshire system (Section 586), where the yeast is periodically carried back to the bottom of the fermenting vessel and well mixed with the wort. In extreme cases it may be necessary to rouse at about 12 hours after collection, and then for 10 minutes every 3 hours until 3 hours before skimming time, as judged by the required fall of gravity. In other cases it may be desirable to rouse at about 24 hours after collection and every 6 hours until about 4 hours before skimming. Much less rousing is required with weakly flocculating yeasts. A single rouse on the morning after collection may be adequate to ensure satisfactory attenuation, or two or three rousings during the course of fermentation may be needed. Yeasts vary so widely in their flocculating properties that the proper course must be decided by observation in each case. If the black sludge is roused in with the early heads, it usually comes up again satisfactorily and can be removed. but care must be taken not to remove yeast with it.

The old-fashioned method of rousing with a bucket, pierced with holes, which was lowered and raised by means of a rope and pulley, was very effective but liable to be a source of infection

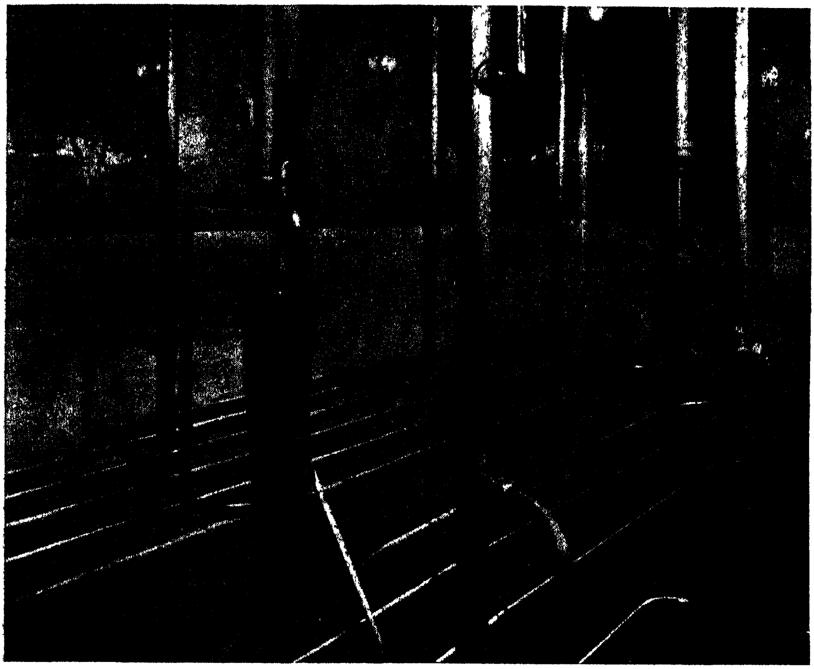

Fig. 162

SCOTT'S CONE ROUSING APPARATUS AND ATTEMPERATORS IN FERMENTING VESSEL (ANSELLS BREWERY, LTD., BIRMINGHAM)

The motor-driven centrifugal pump, now more commonly employed, may be equally open to suspicion, unless great attention is paid to its cleanliness and sterility. It delivers wort over the heads and may fail to submerge a good deal of the yeast in large vessels, unless assisted by drawing the heads under the stream of wort or moving the pump from place to place. An appliance, consisting of a vertical pipe of sufficient length to reach to the bottom of the vessel, with another delivering compressed air to its lower end, is widely used in Scotland. Wort is driven up the pipe and delivered in a stream over the yeast heads. A rapidly rotating, cone-shaped device, patented by W. Scott, is shown in Figs. 158 and 162. This draws up the wort and discharges it over the yeast. A current of compressed air delivered to the bottom of the vessel has a rather different effect, in that it does not increase attenuation by beating in the heads. It may help by dispersing bottom yeast in the wort, but it also tends to arrest fermentation by bringing it to the surface.

Worts are usually attenuated to about one quarter of their original gravity, but greater stability is in some cases attained by attenuating to a fifth, particularly for bottled beers. This may require between 72 and 96 hours from collection, by which time the temperature will have fallen well below its maximum. Skimming usually continues for between 24 and 36 hours, and is terminated when the yeast is judged to be capable of throwing up just sufficiently more yeast to form a protective coating. At this point, the beer appears nearly clear, when observed after drawing aside the yeast cover. It is advisable to keep the maximum temperature as low as is consistent with active fermentation, and to remove the yeast heads as rapidly as possible, in order to avoid the excretion of metabolic products which give the beer the harsh flavour associated with "yeast bite." A lower temperature also favours the retention of gas. The curves in Fig. 150 represent a fermentation in a skimming vessel.

(584) Dropping System.

The dropping system is a development of the skimming system in which the wort is run down to a second vessel for skimming. A moderately fast yeast is usually employed and no further rousing may be required than that caused by transfer from one vessel to the other, though rousing may be adopted, as in the skimming system, if the yeast is of a more flocculent character. In some breweries the wort is dropped at about 24 hours after collection, by which time a good deal of the heavier sediment has settled and is left behind, together with some active yeast and much of the sludge which had risen with the first heads. In other breweries, the dropping time is fixed at about half gravity, or when the frothy head commences to fall from its highest point and temperature has reached its maximum. This is usually between 36 and 42 hours after collection. A rather higher pitching rate than that adopted for skimming squares should be employed, to make up for the inevitable loss at dropping. Skimming is carried out in the dropping vessels, which are situated on the floor below the collecting vessels, while slate yeast backs were formerly placed on a floor below this. The movement caused by dropping rouses the yeast very effectively, but at the same time removes a great deal of the CO_2 already formed.

This and the aeration produced were, at one time, considered as advantages of the system. The view that aeration is an advantage at this stage has probably no foundation in fact, rousing being the most important result of the transfer with the advantage of greater cleanliness secured by leaving the heavier sediment in the upper vessel.

(585) Burton Union System.

The oldest fermentation systems, in which beer is fermented entirely in trade casks or cleansed in these after fermentation in tuns (Figs. 5 and 6, Vol. I), are still practised to a limited extent in some very small breweries or for special local beers, such as the Peeterman of Louvain or Lambic of Brussels, reliance being placed on organisms in the wood of the casks for fermentation of the latter. When such " loose pieces " are employed, the yeast may be added in the pan of the refrigerator or in the fermenting tun. These methods, too wasteful for general use in large breweries, were developed to become the Burton Union system, in which a number of 3½- or 4-barrel casks are fitted on trunnions, below and on either side of a " barm back," into which they deliver yeast and beer through " swan necks," as shown in Fig. 161. One end of the barm back is pierced by a series of cork holes at different levels, through which beer flows to a second, transverse back or " feeder " and thence to the casks through mains which run along the front of each row of casks and are fitted with valves at the feeder and at each cask. Every cask is fitted with a flattened, tinned copper attemperator, and an attemperator coil is suspended in the barm back. The beer is racked through a screw plug cock, which can be raised or lowered into the bottom of each cask to hold back the yeast sediment and delivers to a trough leading to a racking square.

The yeast is of fast, non-flocculating type, and requires no rousing beyond that afforded by the flow of wort into the fermenting vessel at pitching, by that which occurs when the wort is pumped to the union sets and by the movement during cleansing. The fermenting vessels are usually of about 50–60 barrels capacity, and are not fitted with attemperators. Two generally serve one union set. Fermentation continues in them for some 36–40 hours, by which time the wort will have attenuated to between ¾ and ½ of its original gravity and have reached its maximum temperature. It is then pumped to the feeder of a union set, from which it flows to the casks, two or three of which are cut off and kept empty as " cold ale " casks. The fermenting wort at once commences to work through the swan necks into the barm back, carrying yeast with it. This wort and suspended yeast is fed periodically into the feeder and thence back to the casks, which are kept full. In about 15 hours, the beer runs almost clear from the barm back and, at this stage, the yeast which has settled in the feeder is removed, so that clear beer can be fed to the casks. Feeding goes on during 30 to 36 hours, with attemperation in the casks as may be necessary. When flow from the swan necks has become very slow, the valve to the casks is closed and beer draining from the yeast is run to the cold ale casks, which are ultimately racked with the remainder of the beer. The yeast settles down firmly in the barm back, is kept cool by means of the attemperator and finally squeegeed out of the back into yeast containers. The thick middle layer only is used for pitching.

Such complicated plant obviously entails considerable labour for cleaning and involves more waste than the skimming systems. The casks are filled with nearly boiling water for cleaning and sterilisation, and " dollied " round on their trunnions by means of handles provided for the purpose. The backs and mains are cleaned in the usual way, the backs being generally brushed over with bisulphite. Though usually of wood, they are now frequently metal-lined. Large horizontal, aluminium tanks, fitted with a number of swan necks, and 50-barrel vertical glass-lined steel tanks, with a single swan neck, have been installed to minimise the labour and loss, while affording the essential continuous cleansing of the Union system. A typical Burton union room and union set are shown in Figs. 160 and 161, while the application of air conditioning to a union room is illustrated in Fig. 159.

(586) Stone Square System.

The Yorkshire Stone Square system is, like the Union system, characterised by the small capacity of the fermenting vessels, and is practically restricted to Yorkshire, Lancashire and neighbouring districts. The vessels consist of a fermenting square, surmounted by a yeast back, with a manhole in the centre of the separating "deck." The fermenting vessel was originally surrounded by a water jacket, but attemperation is now more generally effected by means of a coil, which may be fixed or inserted through the manhole. The latter is surrounded by a circular curb, about 6 inches deep, to retain yeast and wort in the upper back. The deck or diaphragm is also pierced with two or three holes. A copper " organ pipe " passes through one and reaches almost to the bottom of the lower vessel, for return of yeast and beer. A side pipe is connected to the second and passes obliquely through the jacket for running off " back drink " or yeast. Rousing is an essential part of the process, on account of the very flocculent character of the yeast used. It is carried out by means of a pump through the manhole or connected with a third short pipe through the deck. Racking cocks are fitted through the bottom or side, close to the bottom. In the latter case, a drainage cock is also required. The vessels and jacket were formerly made of stone or slate slabs, but they have in recent years been largely increased in size and constructed of aluminium or stainless steel. The most modern construction is shown in Fig. 164.

Normally a number of squares are filled from a wort main, through branch mains, as in other systems. The wort runs from the upper back through the organ pipe, usually occupying a depth of two or three inches in the yeast back, as well as the lower vessel. Pumping usually commences 24–36 hours after collection, the wort mixing with the yeast in the upper vessel and running back through the organ pipe. Pumping for about 10 minutes at a time is repeated every 2–4 hours until the desired attenuation is reached, or about 6 hours before yeast removal is due to commence. Beer or " back drink " is drained from the yeast, which is then run through the side pipe or otherwise removed from the upper back. Yeast still continues to rise through the manhole for 6 to 18 hours, is drained and collected, the lower vessel being kept full of beer.

Though the yeast is always of flocculating character, there are variations in degree. Some strains rise so completely from the wort that attenuation ceases when pumping is terminated. This must consequently be continued until the final gravity is almost reached. Less strongly flocculating races do not cleanse so fully, and attenuation continues through a few degrees after pumping. The final gravity may be as high as one-half of the original gravity, but attenuation to one-third or one-quarter can be attained if desired.

(587) Fermentation Systems and Beer Character.

The Union and Stone Square systems would probably have gone out of use on account of the labour, maintenance and waste involved, were they not considered to have special claims in respect of the quality or character of the beer produced. They were both developed for stronger beers than the present-day average. The cost of the Union system now almost rules it out of consideration. It does, however, produce a cleaner beer than other systems, and has proved particularly suitable for high quality pale ales. Fermentation in closed vessels prevents disengagement of CO_2 to a considerable extent, and helps to retain volatile, aromatic substances derived from the hops or formed as by-products. Beers brewed on the Stone Square system are full flavoured but, if the final gravity is allowed to remain high, tend to be less biologically stable than more fully attenuated beers. The frequent return of yeast to the beer leads to flavours that are much appreciated in northern districts of England but may, if pronounced, become a yeast bite. This is largely due to beer which has remained in contact with yeast in the upper back for some time, after fermentation is completed, and can be avoided by wasting this beer instead of mixing it with the bulk. The skimming systems are recommended by their simplicity, and their general adoption proves that they are well suited to the production of top fermentation beers. A rather cleaner beer is produced by the dropping system, but the difference should be insufficiently marked to make the complication of the second vessel worth while in most cases.

The correlation between stability and full attenuation, with a low percentage of residual fermentable matter, applies to all fermentation systems. It depends partly on the alcohol content of the beer and probably, to some extent, on other products of fermentation, with the p_H and r_H reached, but the significance of the residual sugars must not be overlooked. It is possible to attenuate a fermentable wort to a low final gravity with a flocculating yeast by long continued rousing, but the marked tendency of the yeast to separate from the wort restricts the attenuation to some extent in practice. A stable final is more easily reached under such conditions with a more dextrinous wort. It is, consequently, usual to adopt a somewhat restrictive mash, that is to say comparatively high mashing temperatures and short stand, in conjunction with Stone Square fermentations or with flocculating yeasts and skimming systems. The advantage of low residual maltose is thereby obtained, though the stability of the beer cannot be quite so great as if this was accompanied by a higher alcohol content.

The full advantage of a low final gravity is secured with a lower mashing temperature and non-flocculating yeast, while the powdery yeast separates more completely than from a fermentation arrested at a higher gravity. The characteristic differences between beers brewed on different systems, thus depend on the mashing methods as well as on the flocculating properties of the yeasts. The preference given to less fully modified malts, from stiffer types of barley, in Stone Square breweries, in comparison with the more fully modified malts used for beers fermented on the Union or other system with non-flocculating yeasts, can be similarly explained.

The degree of attenuation and, consequently, the type of yeast and fermentation system influence the character of the beer in other directions. Hop flavour is brought out more distinctly in strongly attenuated beers. Their flavour is generally "cleaner." A low final gravity does not necessarily mean a thin-flavoured beer, unless the original gravity is rather low, when the production of alcohol and flavouring by-products can no longer compensate for the removal of carbohydrates. In such circumstances the high finals with flocculating yeasts have an advantage. The tendency to formation of non-biological haze is reduced by full attenuation, but the stability of well brewed and attenuated Stone Square beers should leave little to be desired. For export purposes and pasteurisation in bottle, a full attenuation is to be preferred.

All the systems of fermentation described were developed before any consideration was given to the influence of aeration on stability, considered here quite apart from aerial infection, and all appear to contravene the principles more recently established. Oxygen stimulates yeast growth and equally hastens the destruction of beer by invading organisms in the absence of primary yeast. Beer is fully oxidised at pitching and should contain excess of air, which is rapidly absorbed by the yeast in the earliest stage of its growth. After that, some of the constituents of beer, it is still uncertain which, are reduced in the process of fermentation, a change which is reflected in fall of the r_H of the beer (Section 700) from its original value of about 22 to 11 or thereabouts. Yeast can no longer develop when this state is reached, and the reduction is in itself a safeguard against the growth of invading micro-organisms, more effective than attainment of a low p_H (Section 551). The non-biological stability of the beer is also increased by removal of oxygen or reduction, and flavour defects prevented. The result of a normal fermentation is to establish this important safeguard of stability, but the beer is then transferred from vessel to vessel and racked with very little attempt to prevent access of air. A very slight absorption of oxygen suffices to raise the r_H to its original value of 22, and all the benefits of reduction are wasted. The importance of air elimination is realised in connection with bottling, its ill effects being more marked with bottled than draught beer, but they are none the less of considerable moment in casks. Processes have been introduced to prevent access of air in the bottling store, but little has yet been done to eliminate it from cask beer. Developments in this direction will no doubt come in due course, but, in the meantime, such care as is possible should be taken to reduce aeration. A certain amount of oxygen may possibly be necessary to stimulate cask conditioning, but greatest stability cannot result unless reduction to an r_H of about 11 is attained, a condition impossible to realise in presence of air.

YEAST STORAGE

(588) Treatment of Pitching Yeast.

The storage of yeast until it is required for pitching a subsequent brew is of vital importance, because successful fermentation depends on its health, activity and purity. The separated yeast contains a quantity of beer, which varies considerably in different fermentation systems but should, for the sake of economy, be recovered and utilised. Deleterious changes connected with autolysis (Sections 589 and 590) rapidly supervene in the separated yeast and, being accompanied by excretion of carbohydrate and protein degradation products, have a damaging influence on the flavour and stability of the accompanying "barm ale." Yeast storage consequently involves separation of the barm ale before it becomes unfit for blending with beer and preservation of the yeast under conditions which minimise subsequent changes of a deleterious nature.

The question arises as to whether it is desirable to remove the barm ale from the yeast as rapidly as possible or whether a limited time should be allowed in contact with the beer for completion of metabolic processes which accompanied fermentation, but were not brought to a conclusion when the yeast was removed from the fermenting vessel. No clear-cut answer, applicable to every case, can be given to this question. Experience of the behaviour of yeast in subsequent fermentations has shown that it is some cases advantageous, both for the sake of the yeast and of the barm ale, to carry out their separation as rapidly as possible. This applies, more particularly, to yeast that has remained in contact with the beer in the fermenting vessel for comparatively lengthy periods. It has been found, on the other hand, that better results are obtained with yeasts which ferment rapidly and are skimmed off early by allowing a period of rest in contact with the barm ale that may vary from a few hours up to 36 hours but, even with these, the rapid process is successful in many cases and there is little doubt that it is to be preferred with slow working yeasts.

A further question to which no hard and fast answer can be

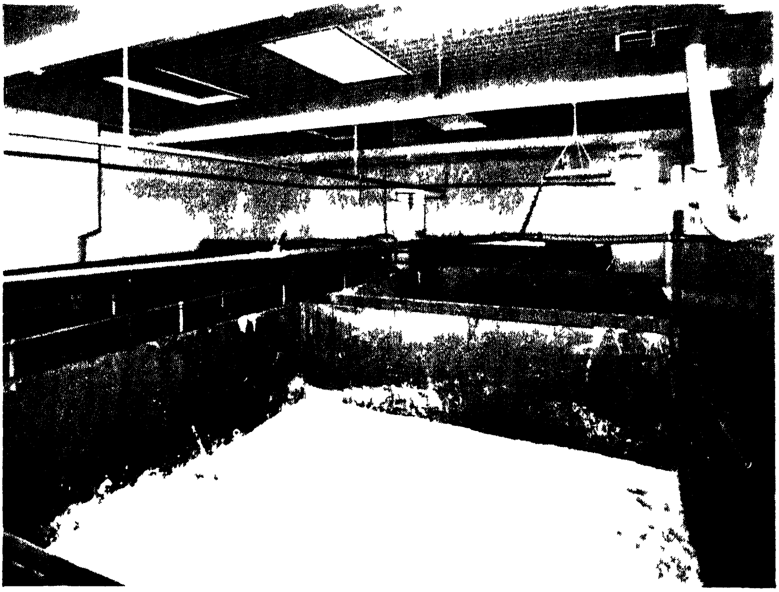

FIG. 163

SLATE VESSELS IN AIR CONDITIONED ROOM
(S. C. WARD AND CO. LTD., SHEFFIELD)

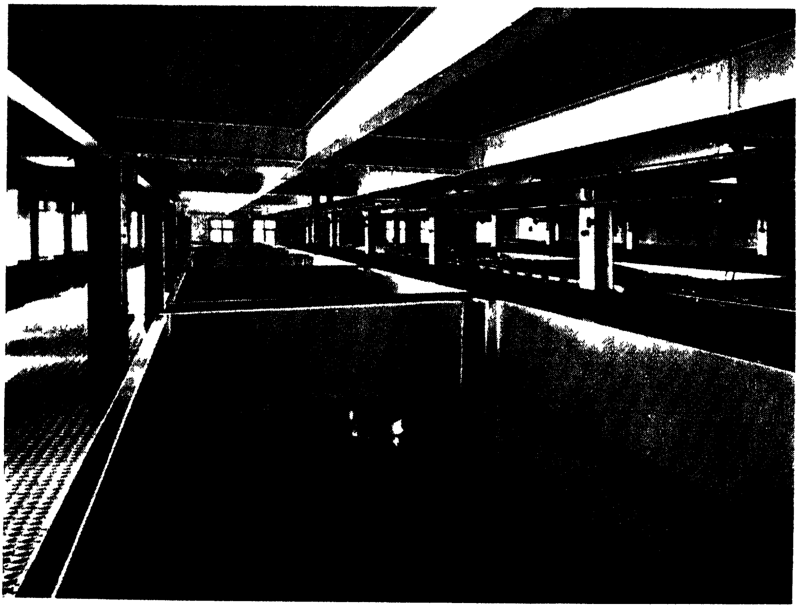

FIG. 164

STAINLESS STEEL VESSELS (JOSHUA TETLEY AND SON LTD., LEEDS)

YORKSHIRE STONE SQUARE SYSTEM

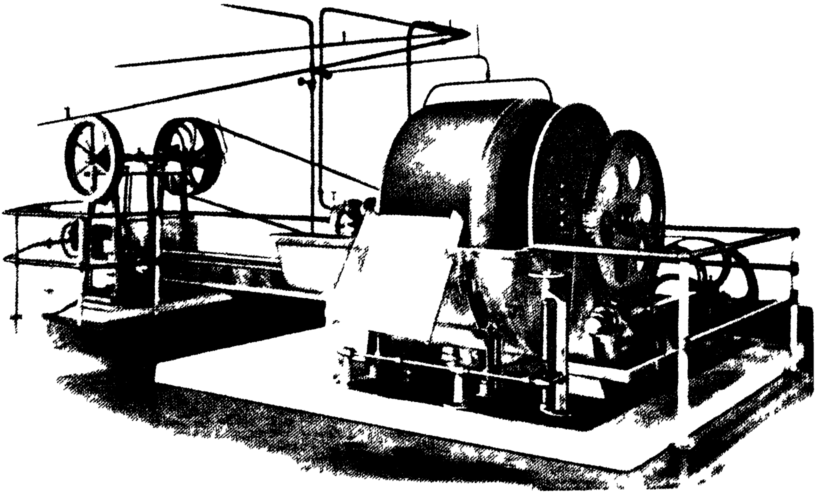

FIG. 165.

FERGUS YEAST SEPARATOR: MEYER SYSTEM.

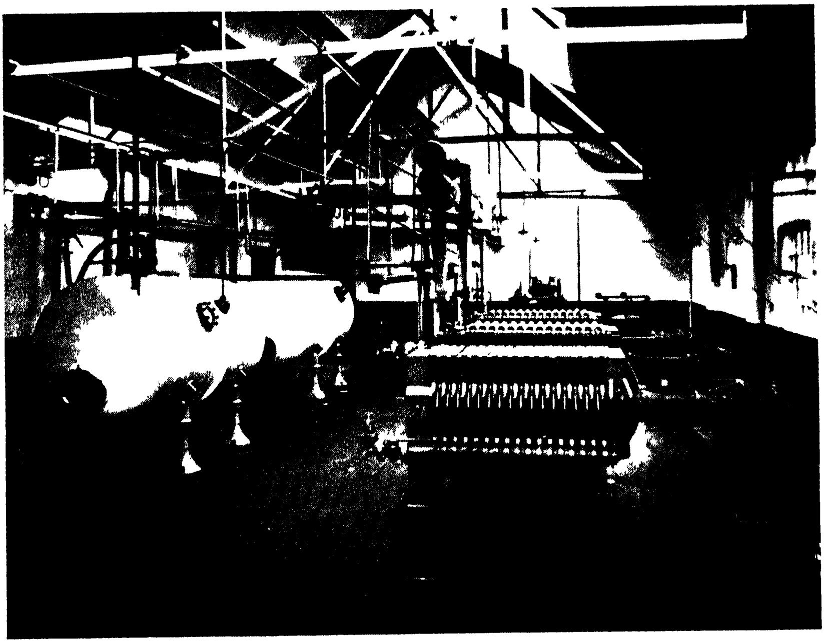

FIG. 166.

SCOTT SYSTEM: COLLECTING VESSELS AND FILTER PRESSES.
(NORTHAMPTON BREWERY CO. LTD., NORTHAMPTON.)

YEAST COLLECTION AND SEPARATION

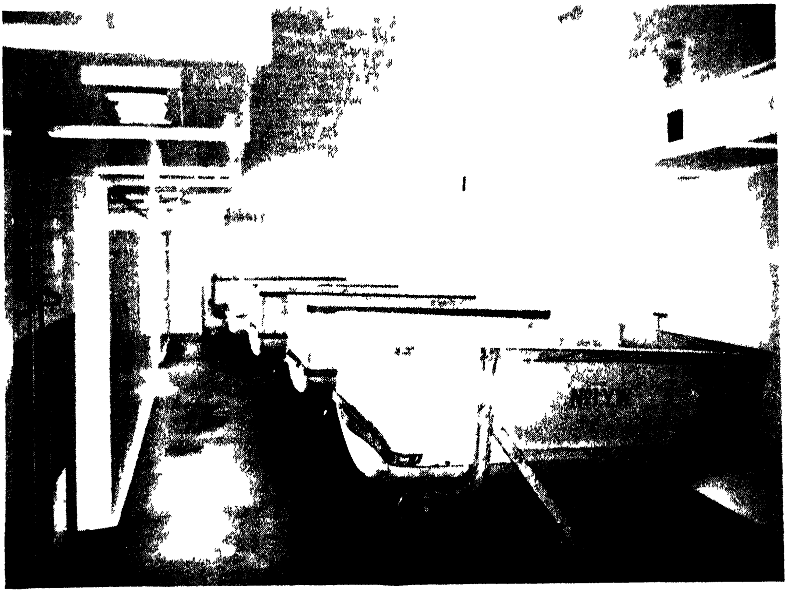

FIG. 167

WITH ALUMINIUM YEAST WAGONS (J. W. GREEN LTD., LUTON)

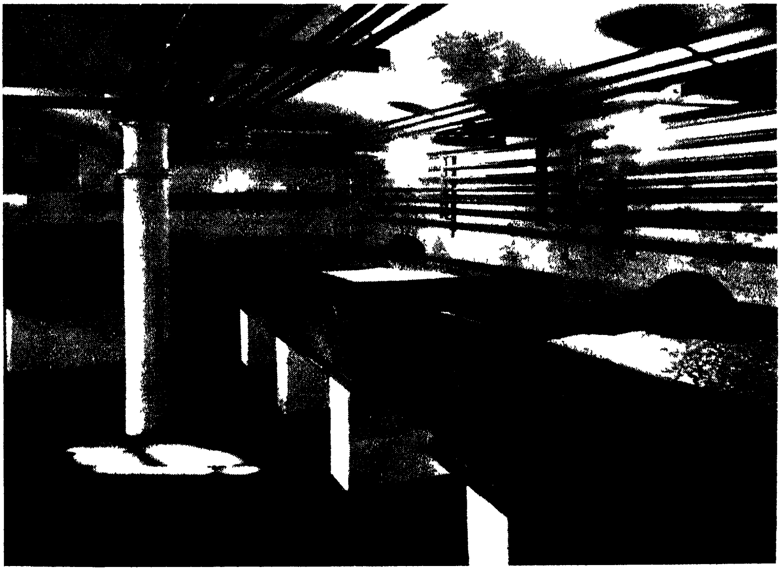

FIG. 168

WITH SLATE SETTLING AND STORAGE BACKS
(JAMES HOLE AND CO. LTD., NEWARK-ON-TRENT)

AIR-CONDITIONED YEAST ROOMS

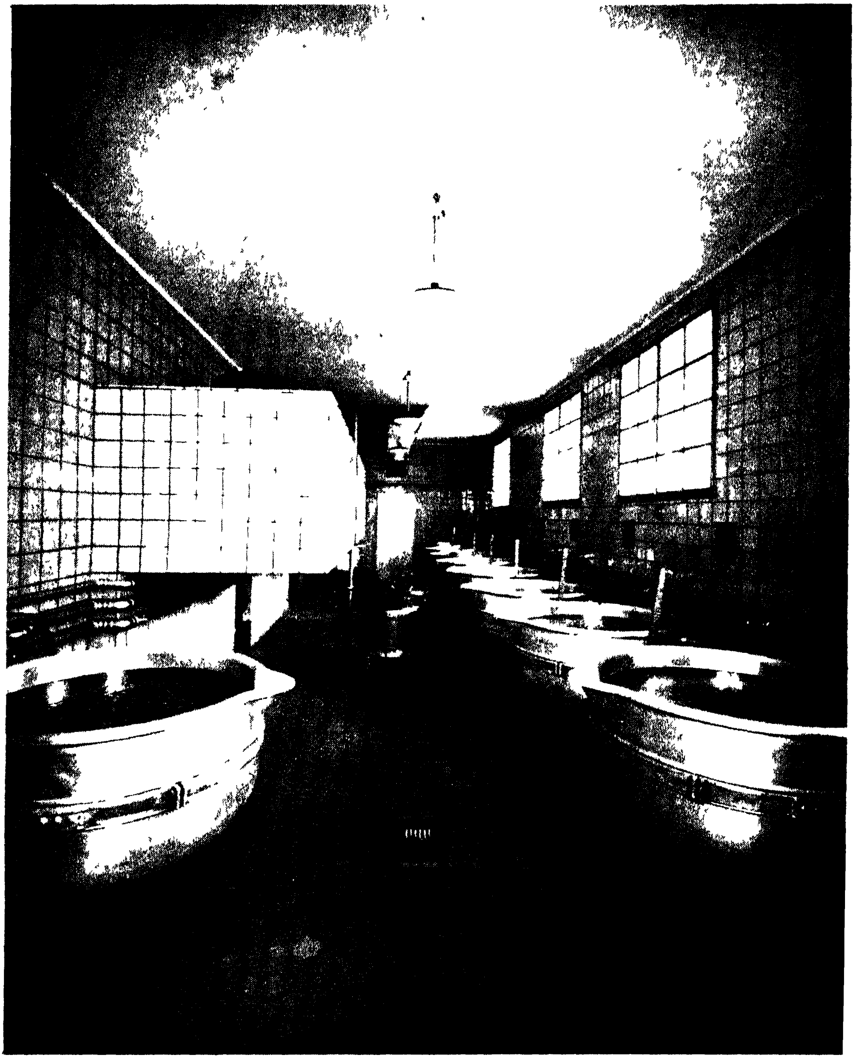

FIG. 169

YEAST WASHING AND STORAGE ROOM (LUZERNER BRAUEREI ZUM EICHHOF, LUCERNE)

Facing page 821]

given is in respect of the time that should elapse between pitching one brew and the next with yeast from the first. This varies in top fermentation breweries, in which the barm ale is drained off as rapidly as possible without suction, from four to eight days or 96 to 192 hours after pitching. Experience points to the necessity for a resting period after the yeast has separated from the beer which is not, in most cases, adequately allowed for in 4 days from pitching. Eight days is longer than is necessary and autolysis may sometimes be noticeable by then unless facilities for low temperature storage are available. As a general rule, six days may be taken as most satisfactory when storage temperatures range between 50° and 60° F. Pressed yeast should be used as rapidly as possible but can, as a rule, be kept for a week at 32° F. without detriment. The least sign of softening is evidence of damage to the cells.

Various methods are adopted for separation of barm ale from yeast. In top fermentation systems, the yeast may be transferred to shallow backs and allowed to rest until the barm ale can be drawn off, or the separation may be hastened by suction, filtration or pressing, giving " yeast drainings " or " yeast pressings," as the case may be. Top fermentation yeasts are rarely washed, but washing is the rule in lager breweries. The yeast which has deposited on the bottom of the fermenting vessel is first washed by decantation and sieving in cold water and afterwards stored under water close to the freezing point in stainless steel, aluminium, or glass-lined vessels, as in Fig. 169.

(589) Changes in Yeast during Storage.

When the normal course of yeast metabolism is upset by lack of nutrients, the cells may continue to live at the expense of their reserve materials. These consist of carbohydrates, largely glycogen, proteins, fats, etc. The breakdown of the carbohydrates resembles ordinary fermentation, in that the decomposition of glycogen results in the evolution of CO_2 and production of alcohol, with small quantities of glycerol and succinic acid. It is consequently referred to as autofermentation and may be observed in yeast immersed in beer or water. Evident dissolution of cell structure occurs on the death of the cells and is marked, in pressed yeast, by softening and, ultimately, liquefaction. Proteolysis plays a large part in this process, which is known as autolysis. Temperature is such an important factor in acceleration of the enzymic processes of autofermentation and autolysis that the practice has been developed of storing yeast at low temperatures, at which the activity of the enzymes is restricted. Autofermentation of glycogen causes a loss of the dry weight of yeast stored in beer and, as this loss is greater than that due to proteolysis, the nitrogen content of the yeast increases, when expressed as a percentage of the dry weight. There is, at the same time, excretion into the beer of products of carbohydrate and protein breakdown. Among these are alcohol and bitter substances that have a deleterious effect on the flavour of the beer. The latter contribute, together with the excretory products of still active cells, to " yeast bite " in beer.

As a result of depletion of the cell contents by autofermentation, there is a considerable extension of the lag phase in subsequent fermentations after lengthy storage. When the yeast is pitched into fresh wort, it must first repair the ravages that have taken place during storage under starvation conditions. The lag phase thus consists of the necessary rebuilding process before fermentation can commence. Tait and Fletcher,[1] who have investigated the changes in yeast during storage, gave the figures in Table 222 for loss of weight and behaviour of fresh yeast and yeast that had been stored 8 days in beer. The yeast lost about 20% of its dry weight during that period and when seeded into fresh wort gained in weight before any measurable reproduction took place. During this quiescent period there was an appreciable assimilation of nitrogen.

TABLE 222.—LOSS OF WEIGHT OF YEAST WHEN STORED IN BEER AND BEHAVIOUR OF FRESH AND STORED YEAST IN FERMENTATION (TAIT AND FLETCHER)

	Original gravity	Grams dry yeast 100 ml.	Millions of cells 100 ml.	Dry weight mgm. million cells
Original fermentation				
Wort at start	1044·2	—	—	—
New beer	1043·5	0·330	4250	0·078
8-days-old beer	1043·7	0·268	4500	0·060

SECOND FERMENTATION WITH NEW AND OLD YEAST

	No. of cells millions 100 ml.		New cells millions 100 ml.		Dry weight yeast mgm. 100 ml.		Dry weight million cells mgm.		Solids fermented gr. 100 ml.		N. assimilated mgm. 100 ml.	
	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Start	920	1800	—	—	94	114	0·102	0·063	—	—	—	—
After :												
5 hrs.	1600	2100	680	300	140	142	0·088	0·068	0·45	0·27	5·8	3·5
9½ hrs.	3240	2340	2320	540	204	188	0·063	0·080	1·11	0·61	11·1	8·4

The accelerating effect of moderate temperatures on the enzymic changes in yeast during storage greatly increases the difficulty of yeast treatment in top fermentation breweries. The yeast head is always a few degrees warmer than the underlying beer in fermenting vessels and the skimmed yeast may have a temperature of 70°–75° F. This tends to increase in a yeast back through continued activity of the yeast, and the heat is not carried away by the small amount of accompanying liquor. Herein lies considerable danger of autolysis, which is caused by temperatures above 77° F. Yeast is a bad conductor of heat and its rate of cooling is further decreased when it is bathed in CO_2, as is always the case with skimmed yeast, which is impregnated with gas to such an extent that 40 to 80% of its volume may consist of CO_2. The first step after skimming is obviously to get rid of this gas, in order that cooling may be unimpeded. It is removed by means of a whisk or rousing paddle when yeast is transferred to a settling back. Some of the effects of temperature are shown in Table 223 with yeast that has been stored at the temperatures given for 3 days.

TABLE 223.—INFLUENCE OF TEMPERATURE DURING STORAGE ON THE BEHAVIOUR OF YEAST IN WORT (TAIT AND FLETCHER)

Storage temperature	Cells per unit volume		Generations	Lag period hours
	Start	After 8 hrs.		
55° F.	0·87	2·61	1·58	1·2
65° F.	1·51	3·55	1·23	2·8
75° F.	1·35	2·35	0·80	4·6
85° F.	1·33	1·54	0·21	7·1

(590) Changes in Pressed Yeast during Storage.

In considering the influence of storage on pressed yeast, note must be taken of interstitial fluid between the cells. Its quantity is small, according to Tait and Fletcher only 0·5 ml. per 100 grams of their hand-pressed yeast, but the contained colloidal and other substances may affect the changes within the cells.

There has been some discussion as to the actual quantity of interstitial liquid in pressed yeast, which usually contains about 70% of moisture. Beetlestone[2] claimed that the actual moisture content of the cells was about 54% and that approximately 26% of the volume of the cake was represented by intercellular spaces. Harden[3] contested this claim for a large amount of interstitial liquid and placed the moisture content of the cells much nearer 70% than 50%. There is considerable difficulty in determining the moisture content of yeast cake on account of the danger of decomposition of cell contents and the difficulty of differentiating between free and combined water.

In some cases, pressed brewery yeast will remain firm for several days at ordinary temperature while, in others, softening very rapidly sets in. Some pressed yeasts can be stored without sensible change in activity for 2 or 3 months at 32° F. but at 52° F. lose their activity and liquefy in less than as many weeks. Tait and Fletcher observed that the life of pressed yeast is prolonged, until the reserves are depleted by autofermentation, by washing before pressing and that, when death did ensue, such yeast did not exhibit the phenomena of liquefaction and autolysis characteristic of untreated yeast. This may be due to removal from the yeast of some toxic substance or to alteration in the nature of autofermentation, so that the poisonous by-products no longer accumulate in the cells. Autolysis is also more rapid if pressed cake is left standing alone than when mixed with water. Schryver, Thomas and Paine[4] were inclined to attribute the difference between washed and unwashed yeast to the removal by washing of wort protein matter which, they suggested, might stimulate the production by the yeast of the pepsin-like enzymes concerned in autolysis. That yeast keeps better in its own pressings, in beer or in water, may on the other hand be attributed to free diffusion of by-products.

When yeast is washed prior to pressing and storage in air, the internal changes are retarded or prevented. The evolution of CO_2 proceeds more rapidly than in unwashed yeast and its continuity indicates that the yeast is living and respiring normally. Alcohol is not formed from the glycogen and the death rate is slow and consistent with the exhaustion of carbohydrates by respiration. Practically no acid is formed and proteolysis is slight. The lag phase in subsequent fermentations with unwashed yeast is probably due to weakening of its vital functions, destruction of zymase and death brought about by accumulation of by-products. In washed yeast, from which by-products that are not reformed have been removed, the cells have gradually depleted their reserves, with consequent reduction in vitality. Hence, when unwashed yeast is placed in wort it must first expel accumulated by-products and then absorb the carbohydrates and proteins necessary to rebuild its starved plasma. Washed yeast, in which there is little or no accumulation of by-products, must also rebuild its depleted plasma before it can commence to grow normally (Tait and Fletcher).

Some of the changes which take place in washed and unwashed pressed yeast when stored in air at 65° F. are shown by Tait and Fletcher's analyses of alcoholic (sp. gr. 0·827) extracts of the yeast after various periods of storage. The dead cells were estimated by the methylene blue method.

TABLE 224.—CHANGES IN UNWASHED (U) AND WASHED (W) PRESSED YEAST DURING STORAGE AT 65° F.
(TAIT AND FLETCHER)
MGMS. PER 1 GRAM

Time hours	Alcohol		Volatile acid		Total acid		Amino-N'gen		Dead cells %	
	U	W	U	W	U	W	U	W	U	W
start	31·5	3·5	0·22	0·08	2·96	2·85	0·43	0·42	5	5
17	49·0	3·5	0·58	0·04	7·92	2·13	0·64	0·54	13	12
24	53·0	3·5	0·43	0·04	8·19	2·22	0·67	0·60	15	10
41	60·0	3·5	0·67	0·04	9·39	2·31	0·76	0·62	26	11
50	52·0	1·0	0·52	0·00	9·81	2·37	0·81	0·65	48	17
72	38·5	0·5	0·74	0·00	—	2·22	-	0·61	47	19
120	11·0	0·0	0·94	0·00	11·04	2·67	1·23	0·81	45	25

During the early hours of storage, the fermentation of reserve material appears to be almost normal, but this stage is usually completed in about 40 hours, after which the death rate rises rapidly and liquefaction becomes noticeable. The pressed yeast cannot dispose of the products of autofermentation and autolysis, the concentration of alcohol rises from its normal of about 4% to a maximum of 8–9%, which may be lethal, but it is probable that death of the cells may be primarily due to accumulation of other by-products, of which acetaldehyde can be detected. There is, in addition, a marked accumulation of acid, which may have a contributory effect in bringing the normal p_H of the cell contents, p_H 6·0–7·0, to that appropriate to enzyme activity. It appears that death of the cells precedes softening or autolysis.

(591) Storage Temperature.

The marked influence of temperature on enzymic activity and changes within the cells raises the question of that which is most favourable for yeast storage and subsequent fermentations. Bottom fermentation yeast is kept very close to the freezing point of water and many top fermentation yeasts suffer no damage and may even be improved in activity if the thick barm is placed in a refrigerated room, a course which is to be recommended with such yeasts if there is any considerable delay between fermentations. This is, however, not always the case and there appears to be an optimum storage temperature, varying with different yeasts, but appreciably higher than freezing point. Heron[5] quotes the experience of several breweries, at some of which the most advantageous temperature was found to be as high as between 50° and 58° F. In others it was between 45° and 50° and in some as low as 40°–45° F. In each case the activity of the yeast was reduced if the temperature rose above the upper limit and rapidly became sloppy if it fell below the lower; 40° seemed to be generally the minimum for the storage of top fermentation yeasts.

These conditions do not hold for pressed yeast which, in many cases, must be stored in a refrigerated room or in the press, cooled by circulation of water or brine. In some cases, particularly with slow yeasts, a temperature of 50° F. in the yeast press is, however, low enough for practical purposes and short storage, but in others it is advisable to use refrigerated water or brine circulation, particularly when storage in the press is prolonged. The need for ample cooling in a press is emphasised by Effront's finding that 2 kilos. of a certain pressed yeast, exposed in layers 37 cm. thick, showed a rise in temperature of 97° F. in 3 hours.

(592) Yeast Backs and Yeast Rooms.

Until comparatively recently, the barm ale was separated from skimmed yeast by draining in some kind of yeast back in which the thick yeast was afterwards stored. The arrangement varied with the fermentation system and might be carried out primarily in the upper deck of stone squares, in the barm back of Union sets, in specially designed backs situated beneath skimming vessels or in shallow backs in a yeast room to which the barm was transported in some form of movable collecting vessel, cask or trolley. This method of draining and storing is still adhered to by many brewers, who claim that it is the best for the health of the yeast and prefer it to any method involving pressing. The backs are usually constructed of slate or aluminium to hold the skimmings to the depth of about a foot. These are frequently placed directly on the floor to accelerate cooling. When the yeast is collected in a sufficiently thick form, as in the Union system, the trolley is simply removed to a yeast room, where it remains until the barm is required for pitching.

The danger of infection in shallow vessels is so considerable that those situated directly under the fermenting vessels or in fermenting rooms have now very largely been discarded and the skimmings are taken as rapidly as possible to an enclosed yeast room, preferably supplied with cooled, filtered air and constructed with every care to avoid possibilities of contamination of the yeast. In modern yeast rooms, the air is reduced several degrees below that at which the yeast is to be stored to hasten the rate of cooling and maintain constant temperature in the cooled yeast. An air-conditioning unit can be used for this purpose or refrigerated, filtered and, if desired, ozonised air may be introduced through a duct about two feet above the floor at one end of the room and evacuated at the other end by a shaft through the roof. The yeast backs are now frequently carried on columns about 3 feet from the floor, which must be made of a smooth, impervious material, such as good quality asphalt. The walls must be smoothly faced with tiles or washable enamel on cement, without angles to collect dirt, and allow sufficient space for easy cleaning. The drainage outlet is at one end of the back, which is slightly sloped in its direction, and provision is made, for instance by a slot in the plug, to drain the beer off slowly and continuously after it has begun to separate.

(593) Separation of Barm Ale by Pressure or Suction.

There is some divergence of opinion among brewers in respect of the advisability of using pressed yeast for pitching. Some maintain that the fermentations suffer thereby, but the majority find equally good results with freshly pressed and liquid yeast or with pressed yeast that has been stored for a reasonable time in a cold room. The ordinary pressure of 40 or 50 lb. per square inch reached in a filter press does not damage the cells and the objections are probably due to the rather rapid deterioration of unwashed pressed yeast when stored at ordinary temperatures, compared with yeast stored in its own barm ale, and the more or less prolonged lag phase in fermentations in which such yeast is used. On the other hand the advantages of rapid separation of yeast and beer can only be secured by some method of filtration. It is usual to reduce the pitching rate with pressed yeast by about 25% from that required with thick barm from which the barm ale has been drained.

The Johnson barm press, operating with a pump which forces the yeast into cakes between plates and filter cloths, is widely used, but speed of filtration is limited by difficulties in pumping thin barm, full of CO_2. Some brewers, who prefer to pitch with liquid barm, make use of the press for excess yeast only and for sediments from fermenting vessels. The quantity of beer reclaimable from yeast varies considerably. In many cases it is between 2 and 3% of the volume of the beer from which the yeast was skimmed. A limit of 3% is set by the British

Excise authorities to the quantity that may be returned to gauged fermenting vessels. Barm ale not immediately returned to the beer should be pasteurised before use.

Suction systems are equally satisfactory for separating barm ale from yeast. Well-known plants devised on this principle are those patented by Meyer[6] and by Littleton.[7] The former consists of a rotating drum, covered with a filter cloth. The yeast is delivered into a tank, in which the drum revolves, while the barm ale is sucked through the filter cloth by a vacuum set up in the drum. The outer layer of dried yeast is removed from the drum by means of a scraper, tendency to break the vacuum being prevented by spraying the incoming yeast over the drum. The Littleton plant takes the form of a large shallow trough on legs, like a table. It is fitted with a perforated plate at about half its depth. This is covered with a filter cloth, clamped to the wall of the trough. The yeast is delivered on the filter cloth and sucked dry by reduced pressure in the chamber beneath.

(594) The Scott Process.

This process for dealing with yeast skimmed from fermenting vessels, devised by W. Scott,[8] is based on the principle that the more rapidly the small quantity of fluid mixed with the large quantity of yeast is separated the better, because the quality of both yeast and beer, after separation, gain thereby. The difficulties associated with pumping freshly skimmed yeast into a press and of cooling collected yeast are both got over in the type of press adopted. The plant, as illustrated in Fig. 170, consists essentially of an aluminium cylindrical yeast-receiving vessel, which may be mounted on a trolley or slung from a runway, according to exigencies of space, and a yeast press operated by compressed air which works satisfactorily, even with the thinnest skimmed yeasts.

The yeast is collected in the aluminium receiver from the fermenting vessel, being removed from the latter by any ordinary method of skimming or, preferably, by air suction, for which the receiver is connected to a vacuum pump. The receiver is afterwards removed to the yeast press, to which it is connected by a short length of rubber hose. The required pressure, which may be 20 to 40 lb. per square inch, is put on the yeast receiver by means of a water-cooled air compressor. The skimmings are, thereby, forced into the press and the filtered barm ale returned direct to the fermenting vessel from which it came. They can be raised if necessary by the air pressure to a higher floor or delivered into a barm ale receiver for conveyance to any required place in the brewery. The pressure provided by compressed air prevents any irregular shocks to the yeast, such as may be given by a pump. Cooling is effected by chilled water circulation within the hollow plates of the press and, since the cakes of yeast formed between the plates and cloths are only 1½ inch thick and none of the yeast is more than ¾ inch from the cooling surface of the plates, the cooling is very rapid. The actual collecting and pressing plant should be supplemented by a rotary cloth-washing machine and a hydro-extractor, in which the cloths are so rapidly dried that only one set of cloths is required. When the filters are in continuous use, as they frequently are, it is only necessary to clean and sterilise them once a week. This can be effected by pumping boiling water through the entire circuit.

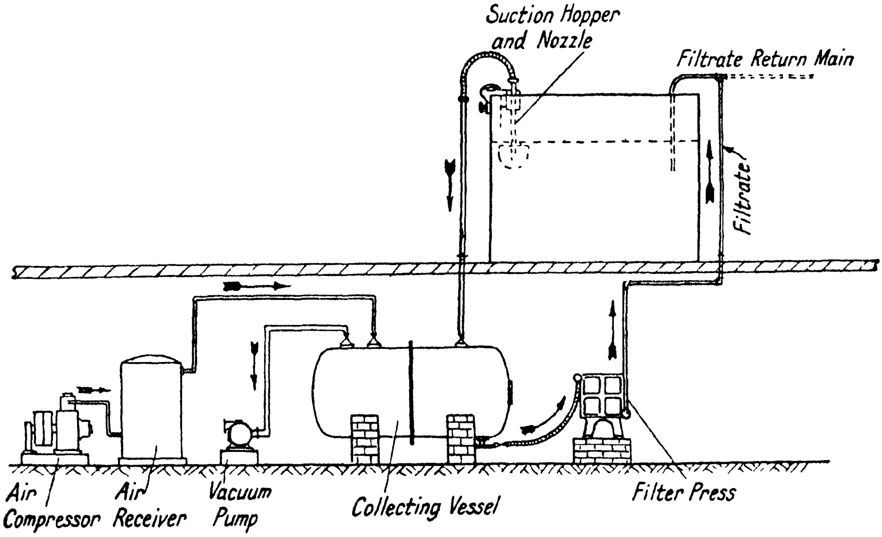

FIG. 170

SCOTT'S YEAST-SEPARATING SYSTEM

The advantages of this system are that freshly skimmed yeast can be handled immediately, that the barm ale is separated without loss and so rapidly that its flavour is very little influenced by excretory products, that the yeast and beer are completely protected from contamination during the process and that the yeast can be rapidly cooled to any desired temperature. The plant takes up little space and is very flexible, being adaptable to practically any system of fermentation or arrangement of plant and the activity of the yeast is unimpaired.

The temperature to which the yeast should be reduced varies considerably with different types of yeast. Slow yeasts, such as those from the Yorkshire system, need rarely be cooled lower than about 50° Fahr. but it is desirable that quick yeasts should be cooled almost to the freezing point of water. Differences of opinion have arisen in regard to the manner of storage of the pressed yeast, but these are probably influenced to some extent by the type of yeast used. Some brewers find that perfectly satisfactory results are obtained by keeping the yeast in the press at a constant low temperature. Others prefer to remove the cake from the press immediately, break it into small pieces and keep it on trays in a cold room or refrigerated cabinet until required. If the yeast is subject to rapid autolysis, it should be washed in cold water as quickly as possible and then dealt with in one of the ways mentioned. A special press is made for this purpose, each cake being thoroughly washed immediately after pressing by passing chilled water.

(595) Barm Ale.

The composition of barm ale differs very considerably from that of the beer from which it was derived, particularly if the skimmings are allowed to stand for any length of time before the yeast and beer are separated. This is shown for original gravity, p_H and ash in Table 225. It will be noticed that these gradually increase with the time the yeast remained in contact with the beer before pressing. The reduction of acidity is probably due to transfer of secondary phosphate to the beer.

TABLE 225.—CHANGES IN COMPOSITION OF BARM ALE WITH TIME OF CONTACT BETWEEN YEAST AND BEER
(HARMAN AND OLIVER)

Time	O.G.	p_H	Ash % in pressings	Ash % in yeast
At skimming	1047·0	4·15	0·205	8·3
6 hours	1055·3	4·60	0·265	7·98
24 hours	1060·1	5·25	0·29	—
48 hours	1061·0	5·65	0·298	7·40

The change in the composition of the barm ale is due to excretion from the yeast of alcohol and other products of auto-fermentation and autolysis, formed after the yeast has been removed from contact with the beer in the fermenting vessel. Among them are protein digestion products which may have a very deleterious effect on the flavour of the beer. The bitterness of barm ale may also be increased to some extent by extraction of hop resins adsorbed on the surface of the yeast. The enzymic changes in the yeast cells and transfer of their products to the barm ale are accompanied by a loss of yeast dry weight as shown in Table 226, where the increase of nitrogen in the barm ale is also shown. This Table also shows how greatly they can be reduced by rapid separation of the yeast from the accompanying beer. It is possible by rapid pressing or filtration to reduce the excretion to very small dimensions.

TABLE 226.—COMPARISONS BETWEEN DRAINED AND FILTERED BARM ALE (FULLER AND MORITZ[10])

	P_2O_5 gr. 100 ml.	Nitrogen gr. 100 ml.	Alcohol % by weight	Specific gravity	Solids gr. 100 ml.
Drainings	0·029	0·567	4·312	1010·06	2·605
Pressings	0·022	0·483	3·157	1015·00	3·886
Beer at skimming ..	0·019	0·326	2·826	1013·66	3·538

A very noticeable difference between the beer and barm ale is to be found in the original gravity, as determined by the distillation process and official tables; the difference varies in different systems of fermentation as well as with the time of contact with the yeast. It is very much smaller when the yeast has been allowed to remain in contact with the beer in the fermenting vessel until its metabolic processes are more advanced, as shown in Table 227 for the stone square and bottom fermentation systems.[11]

TABLE 227.—ORIGINAL GRAVITIES OF BEER AND BARM ALE FROM DIFFERENT FERMENTATION SYSTEMS

System	Union		Skimming		Dropping		Stone square		Lager	
	Beer	Barm ale	Beer	Barm ale	Beer	Barm ale	Beer	Barm ale	Beer	Barm ale
Original gravity	1055·5	1063·1	1043·2	1055·8	1049·4	1057·0	1046·4	1047·6	1045·9	1045.4
Density of beer ..	1014·4	1005·6	1012·9	1005·4	1012·4	1004·0	—	—	1006·9	1004·6
Alcohol % by wt.	4·20	5·99	3·09	5·33	3·79	5·50	3·23	4·07	4·05	4·15

(596) Summary.

The various fermenting systems used for top fermentation beers differ in the means adopted for keeping the yeast in the wort during the period of its greatest activity, and for removing it from the wort afterwards. These differences are dictated largely by the flocculating properties of the yeast. A strongly flocculating yeast is most adequately dealt with by the Yorkshire system which provides a means for returning the rapidly separating yeast to the body of the wort by frequent pumping and mixing with wort in the upper deck of the " stone squares." The Burton Union system deals more efficiently with a rapidly fermenting, non-flocculating yeast than any other plant. The Skimming system is what might be called an all-purposes process, since it is used satisfactorily with all types of yeast, some brewers preferring a Burton and others a Yorkshire strain. The details of operation must, however, be varied strictly in accordance with the type of yeast employed. Too little rousing with a flocculating yeast will inevitably result in defective attenuation, and too much rousing of a non-flocculating yeast will impair cleansing. Many apparent defects in fermentation arise from failure to make the process approach as closely as possible to that which best suits the yeast. A good rule is never to rouse more than is necessary to maintain a normal rate of attenuation. A second rule is to allow the heat generated by fermentation to raise the temperature of the wort gently and regularly, never to allow the rate of fermentation to become excessive by too rapid rise of temperature or to check the yeast abruptly by cooling before its work is properly accomplished.

Given these conditions a healthy yeast will behave in a normal manner. It will attenuate the beer to a point where its stability is assured, and fortify this stability by reduction of its p_H and r_H to low values. Yeast, however, requires further consideration for maintenance of its activity at the highest point of efficiency. The composition of the wort must provide all necessary nutrients in adequate quantity, together with sufficient fermentable sugars. It must be saturated with oxygen during cooling in order that the yeast may find enough to supply its needs in the early stages of fermentation, and so shorten the lag phase. Skimming should not be delayed when the yeast head has formed. Its high temperature accelerates excretion of autolytic products, which may easily cause a definite " yeast-bite." The yeast must be dealt with kindly from the moment it is skimmed from the wort until it is again used for pitching. This means, in general, a fairly rapid removal of the accompanying barm ale, with reduction of temperature to that most suited to the particular yeast. Some variation exists with different strains in this respect, but pressed yeast is always best kept near the freezing point of water.

Skimmed yeast is usually at its best 5 or 6 days from pitching or about 2 days after skimming. Products of fermentation diffuse into the barm ale. The glycogen reserves are also progressively depleted, so that the start of fermentation with older yeast is delayed while the deficiency is made good. Pressing prevents diffusion. Toxic products accumulate and the death rate is more rapid than in liquid barm at ordinary temperatures. Autolysis of dead cells, accompanied by liquefaction and development of a musty odour, rapidly sets in. The consequent objection to the use of pressed yeast is overcome by storage at 32° F. The browning of yeast stored on trays in a cold room is apparently largely due to drying. Normal respiration, with depletion of carbohydrate reserves, takes the place of autolysis if the yeast is washed before pressing or in the press. It will then remain sound for about a week at 65° F.

The difficulty of maintaining a supply of healthy pitching yeast when only low gravity beer is brewed may be overcome by suitably spacing stronger all-malt brews and blending before racking with partly fermented sugar solutions of low gravity. Invert sugar and certain less fermentable, proprietary sugars are used in this way in the so-called "process beers." For example, 50 barrels of beer at 1051 original gravity may be blended with 100 barrels of sugar solution of 1012 to give a beer of 1025 sp. gr. The sugar is boiled in the copper with a little hops and fermented at about 60° F. for 3 or 4 days with ½ lb. of yeast per barrel. Saturation of the sugar solution with CO_2 prevents flattening and a full flavoured beer is obtained. A racking back or other vessel must be provided for blending, and special arrangement made with the Excise under existing regulations for fermenting under 1027 sp. gr., in order to avoid excessive duty on the blend.

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CHAPTER XXXIV

BOTTOM FERMENTATION SYSTEMS AND BULK CARBONATED BEERS

PRIMARY FERMENTATION

(597) Continental Lager Fermentation.

The process generally employed in Continental lager breweries comprises primary fermentation in open vessels, or in closed vessels adapted to the collection of CO_2, followed by prolonged storage in large casks or tanks in cellars at about 32° F. for after-fermentation, conditioning, clarification and maturation. There is a fairly wide variation in the range of primary fermentation temperatures in different breweries or for different beers in the same brewery. Low temperatures cover a range of from 5° C. at pitching to 8° C. at the height of fermentation (41°–46·4° F.). Higher temperature ranges are about 6°–10° C. (42·8°–50° F.) and, in some cases 7°–12° C. (44·6°–53·6° F.). Low temperatures give beers of finer quality and fuller flavour. The higher temperatures give a more vigorous fermentation with reduced tendency to yeast deterioration and wild yeast development. Pale beers are frequently pitched and fermented at rather lower temperatures than dark beers in the same brewery. Eleven days may be required at the lower temperatures, sometimes 12 or 14 days are allowed, while 7 to 9 days suffice at the higher temperatures. The figures in Table 228 exemplify attenuations during primary fermentation and storage, the apparent limiting attenuation found experimentally being given for comparison.

TABLE 228.—PRIMARY AND AFTER-FERMENTATION OF LAGER BEERS

	Sp. Gr.		Saccharometer Extract% Plato		Apparent Attenuation per cent.	
	pale	dark	pale	dark		
Original Gravity	1048·4	1048·4	12	12	—	—
End of Primary Fermentation	1016·9	1021·3	4·3	5·4	64·2	55·0
End of Storage (Racking) ..	1012·1	1017·7	3·1	4·5	74·2	62·5
Apparent Limiting Atten. ..	1010·9	1015·3	2·8	3·9	76·7	67·5

The difference in the apparent attenuation at the end of the primary fermentation and at racking depends, largely, on the composition of the wort and the character of the yeast. In general, a difference of about 10%, corresponding with about 4°–8° of gravity with a beer of 1048 sp. gr, is required to produce the necessary condition. Differences of less than 5% may prove too small, in which case it would be necessary to kräusen or add fermenting wort to activate the fermentation. The range required also varies with the temperature of storage and its duration, and is usually rather less with dark than pale beers. The amount of fermentable matter at the end of primary fermentation must not be so great that fermentation in the storage tank commences too rapidly, nor so little that the after-fermentation lags or is too weak to produce the necessary amount of gas for adequate condition.

An essential condition of a well balanced fermentation is that the primary fermentation should slow down and come to an end while sufficient fermentable extract still remains for a satisfactory after-fermentation in the storage tanks. The primary fermentation is judged to be finished when the saccharometer reading falls by no more than 0·15–0·2% Plato (0·6°–0·8° of gravity) in 24 hours and a firm cover of yeast has formed, beneath which the beer appears black or nearly so, according to the flocculating properties of the yeast. The latter should then, for the most part, have settled firmly on the bottom of the vessel, leaving only sufficient in suspension to carry on the after-fermentation. As this time approaches, the beer is examined from time to time in a small glass and the appearance of the break noticed. The quantity of yeast required in the beer at transfer to tanks varies in different circumstances. More yeast is required if the storage cellar is very cold, and the beer is transferred rather "green" in order that the fermentation should start again reasonably quickly, though this must not be too energetic. A clearer beer is preferable when the cellar is warmer, when the beer has not been fermented very fully and contains plenty of fermentable sugar, or when a fully fermented beer is to be stored for a long period.

Naturally fermented lager beers are not usually fermented so fully as top fermentation ales, but very different finals are aimed at with different types of beer. A pale beer is taken to a lower saccharometer reading than a dark beer of the same original gravity, while export beers and those which are to be pasteurised are fermented more fully than quick trade beers. Low gravity beers, 1040 and under, are not fermented so fully as beers of 1045–1055, while strong beers of 1060 sp. gr. and upwards are again usually fermented to a smaller extent. Yeasts of good flocculating properties are used for beers that must clarify comparatively rapidly in storage for draught trade. Powder yeasts are preferred for long stored beers, with which full attenuation and great stability are required.

Temperatures during primary fermentation are largely controlled by refrigeration of the fermenting cellar itself. The rooms are kept at about 5°–6° C. (41°–43° F.), the rise of wort temperature being thereby steadied and kept within the limits necessary in healthy fermentations. This temperature is sufficiently low to cause the slow fall in wort temperature desirable after the first energetic stages of fermentation have passed, but the final cooling required to assist in the sedimentation of yeast and bring the beer to the best condition for transfer to the storage cellar is carried out by attemperators, through which refrigerated brine, chilled water or, in some cases, 20% alcohol, is circulated.

(598) Course of Bottom Fermentation.

The pitching rate is commonly calculated on the basis of about 1 litre of thick liquid yeast for 50 kgm. of grist, equivalent to about 1½ lb. per barrel. The yeast is mixed with 10 to 15 times its volume of wort at between 12° and 15° C. (54°–59° F.) and thoroughly roused or aerated to give fermentation a good start. It is usual to collect and pitch the wort in the fermenting vessels but, in some breweries, it is pitched in special collecting vessels, from which it is run to the fermenting vessels after about 24 hours, leaving behind a good deal of sludge and some hop resin. The collecting vessels do not contain attemperators, but the fermenting vessels are fitted with fixed or movable coils. In some breweries the yeast is added slowly to the wort in the pan of the refrigerator.

Worts are roused during the first day or two to increase the rate of fermentation and yeast reproduction but this, if excessive, is liable to cause progressive deterioration of the yeast. Yeast cells have been found to become smaller, with greater granulation and smaller vacuoles in fermentations that were vigorously roused at pitching and once daily during the first four days. Attenuations become less complete after successive fermentations and excessive aeration would appear to be responsible for some cases of yeast enfeeblement and unsatisfactory behaviour in the storage tanks (F. Windisch[1]). The stimulating effect of rousing was attributed in Section 562 mainly to keeping the yeast in suspension, but Windisch held that the increased rate of fermentation was partly due to the accompanying aeration and accelerated reproduction. His experiments on the effects of oxygen showed the limitations of aeration in increasing fermentative activity. This involves a compromise in brewery practice in order that the activity of pitching yeast should not be sacrificed to reproduction, which must, however, be adequate.

Bubbles of gas form at the sides of the fermenting vessel in the course of the first 15–20 hours and gradually cover the surface of the wort, forming a thin covering with some hop resins and other colloidal matter, which is skimmed off. The specific gravity of the wort falls and its temperature rises, slowly at first, as the white curly or " cauliflower " head of the " low kräusen " forms. This should cover the entire wort surface by the end of 36 hours and brings more hop resins with it. The most active stage of fermentation follows and is marked by the " high kräusen " or " rocky heads," but these do not rise nearly so high as in top fermentations. The varying appearance of the heads is shown in Figs. 172 and 173. The high kräusen may last for 48–72 hours, afterwards falling back to form a firm covering. It marks the most active period of yeast reproduction, with greatest attenuation, the temperature rising to its maximum. This temperature is held for a day or two, after which the wort is gradually cooled back at the rate of about 1° C. in 24 hours to 5° or 6° C. (41°–43° F.) and then run to the storage tanks. Typical figures for a 7-day and 11-day fermentation are given in Table 229, with a diagram of the latter in Fig. 171. Yeast reproduction is usually

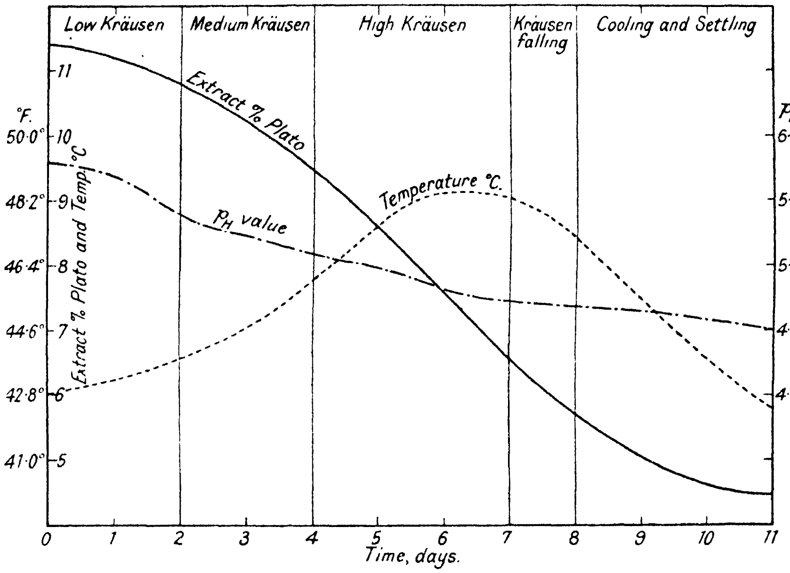

FIG. 171

DIAGRAM OF BOTTOM FERMENTATION

three- to four-fold, but only about twice the weight of pitched yeast may be recovered for further use, allowing for the upper and lower layers of sedimentary yeast which are not used for pitching, and the loss in washing.

TABLE 229.—PALE LAGER FERMENTATIONS

Days	7 days				11 days			
	Extract % Plato	Temperature C.		p_H	Extract % Plato	Temperature C.		p_H
		a.m.	p.m.			a.m.	p.m.	
Pitch	11·0	—	6·5°	5·5	11·4	—	6·0°	5·8
1	10·7	7·0°	7·4°	5·1	11·2	6·2°	6·3°	5·7
2	9·7	8·1°	8·4°	4·8	10·8	6·6°	6·8°	5·4
3	7·8	9·2°	9·2°	4·6	10·2	7·0°	7·5°	5·25
4	5·4	8·5°	8·0°	4·5	9·5	7·8°	8·1°	5·1
5	4·3	7·3°	6·6°	4·3	8·6	8·6°	9·0°	5·0
6	4·0	5·6°	5·0°	4·3	7·6	9·1°	9·0°	4·8
7	3·8	5·0°	—	4·3	6·6	9·0°	8·8°	4·75
8	—	—	—		5·7	8·4°	8·0°	4·7
9	—	-	-	-	5·1	7·5°	7·0°	4·65
10	—	—	-	—	4·6	6·6°	6·3°	4·6
11	—	—	—		4·5	5·8°	—	4·5

(599) Fermentation at Higher Temperatures.

Higher fermentation temperatures with bottom yeasts have been widely used, particularly in America, since the advantages of the more vigorous fermentation and reduced time of contact with yeast were pointed out by R. Wahl and M. Henius. Pitching temperatures are between 7° and 9° C. (44°–48° F.), the wort rising to between 11° and 13·5° C. (52°–56° F.). When the high kräusen commences to fall back, the wort temperature is at first allowed to drop spontaneously but, when fermentation is practically completed, the wort is rapidly cooled with brine to about 4° C. (39° F.). Attenuation is carried substantially to the limit, as no further fermentation is required in the storage cellar, the beer being carbonated before racking or bottling. The yeast settles well and the beer is run to the storage cellars in brighter condition than is usual with less attenuated beers in lower temperature fermentations. The yeast obtained is healthy and free from contamination with other organisms, while the beer is less liable to take a flavour from it on account of the reduction in time to 7 days. This system has been successful in the production of fully attenuated, stable beers, particularly such as are brewed with a high proportion of maize or rice and pasteurised in bottle.

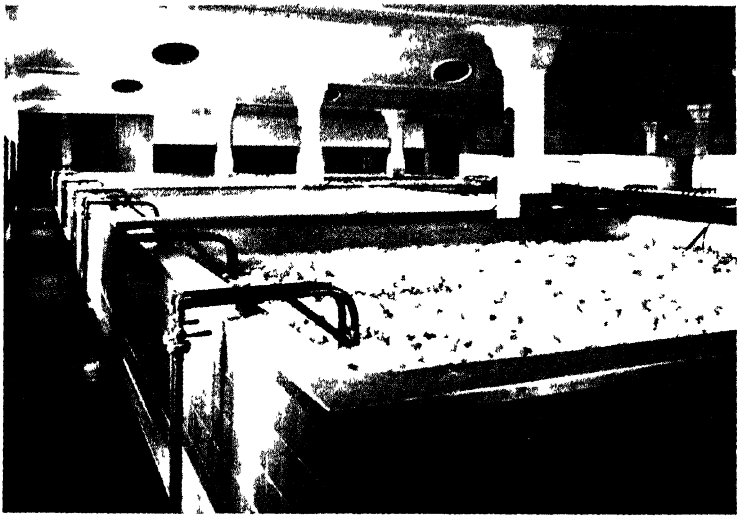

FIG. 172

LOW KRAUSEN

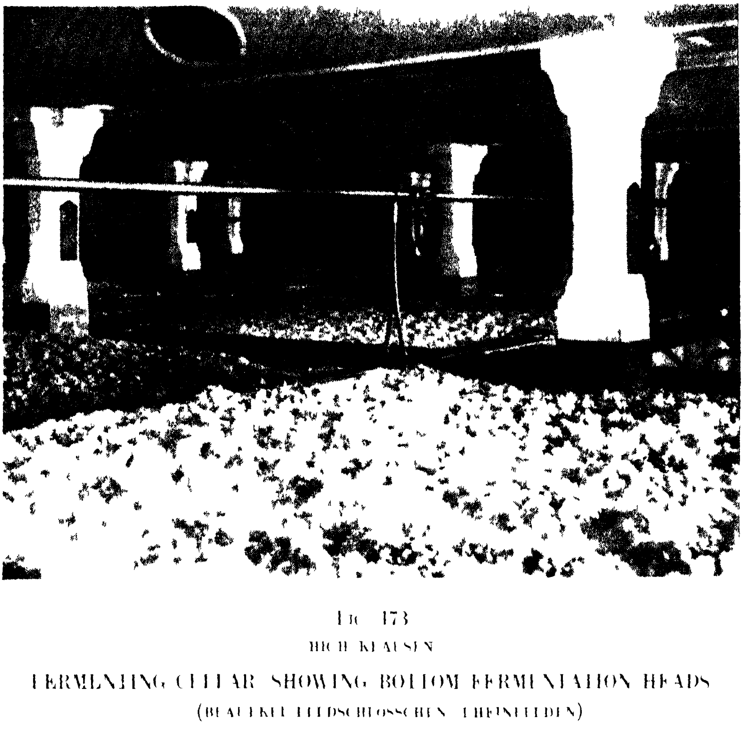

FIG. 173

HIGH KRAUSEN

FERMENTING CELLAR SHOWING BOTTOM FERMENTATION HEADS

(BRAUEREI FELDSCHLOSSCHEN, RHEINFELDEN)

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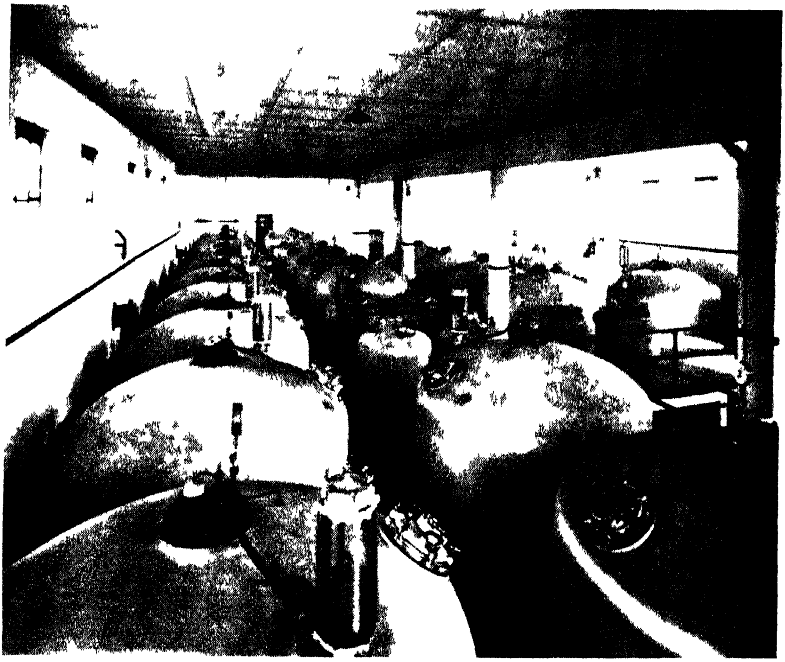

FIG. 174

FERMENTING VESSELS

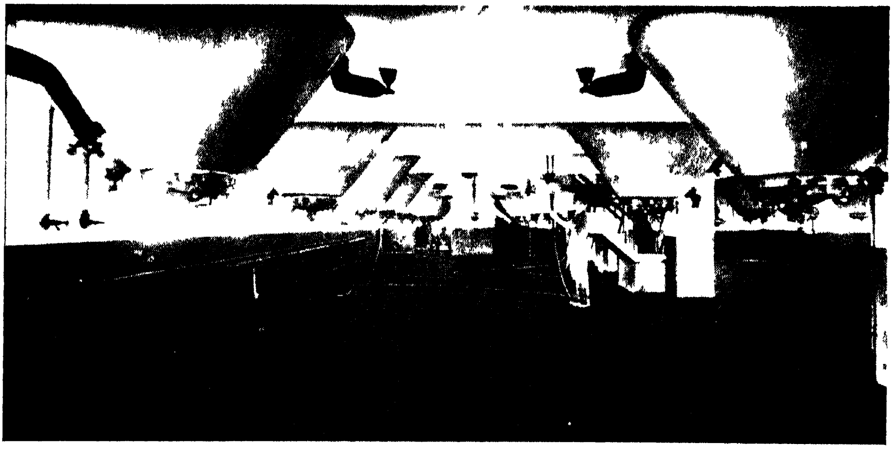

FIG. 175

LOWER PART OF FERMENTING VESSELS

NATHAN BREWING SYSTEM

(RICHMOND BREWERY, MELBOURNE)

(600) Fermentation in Closed Vessels.

The recovery of CO_2 by fermentation in closed vessels is more convenient with bottom than top yeasts, on account of the smaller heads, and offers several advantages quite apart from this. Fermentation under fairly high pressure appears to have no harmful effect on yeast. Lieske and Hofmann [2] found that experimental fermentations were arrested when the pressure rose to 38–40 atm., but this might be attributed to high acidity produced by the CO_2 (Windisch[3]). Stockhausen and Windisch's [4] experiments under pressures up to 1 atm. showed a slight retardation of fermentation but no sign of toxic effect on the yeast, but, on the contrary, benefit to it. In any case, pressures would be small in brewery fermentations and comparative trials have shown that the yeast was more active after fermentation in closed than open vessels and that the beer was better. In addition there are the advantages of protection from air-borne infection and a more healthy atmosphere in the fermenting cellar.

(601) The Nathan System.

Fermentation in closed vessels has been carried to an advanced stage in the Nathan system of brewing, in which a beer of lager type is produced in 12 days from mashing to racking. Mashing and boiling are carried out in the ordinary manner, but the system comprises a special cooling and settling plant (Section 511), from which sterile wort is delivered to the fermenting vessels. These are usually constructed of aluminium, are completely enclosed, have conical bottoms, as shown in Figs. 174 and 175, and are provided with cooling jackets, through which a refrigerated alcoholic liquid is circulated. They are sterilised with alcohol vapour before being filled with wort. The necessary amount of yeast is forced in by sterile air or CO_2 from a yeast storage vessel, similar in shape, but of smaller size than the fermenting vessels, and thoroughly mixed with the wort by the gas current. The initial temperature is 3°–4° C. (37°–39° F.) for pale beers and 4°–5° C. (39°–41° F.) for dark. Fermentation is consequently slow and 20 hours may elapse before CO_2 issuing from the top of the vessel is free from air and fit to be passed to the gas-holder. The temperature is kept low and fermentations regulated by agitating the beer with CO_2, which enters at the base of the cone in a finely divided state through an artificial porous stone.

The gas issuing from the fermenting vessel is collected after purification by passage through water, potassium permanganate, sulphuric acid and soda, and compressed to between 2 and 3 atmospheres for future use. About 6½ lb. of liquid CO_2 can be obtained from a barrel of beer of 1049 gravity. At the yeast settling period the beer is subjected to a CO_2 pressure of about 0·5 atm. to limit further movement. The cooling jacket of the cone is opened to assist in settlement of the yeast so that it can be more readily forced to the yeast vessel. After the yeast has been withdrawn, the beer is allowed to remain in the fermenting vessels for the maturation process.

The character is obtained by removing the flavouring substances of new beer by washing with CO_2, rather than by development of other substances during the period of storage. The "washing" process consists in passing a current of finely divided CO_2 through the porous stone and into the beer for 24 or 36 hours. The beer is then cooled to 1°–0° C. and saturated with CO_2 under a pressure of 0·35 to 0·4 atm. A final stand of 12 hours is allowed before racking or bottling. The advantages of time and protection offered by this process are obvious. Beer of good quality is produced, but the storage must be prolonged if it is necessary to secure the greatest non-biological stability by precipitation of haze-forming substances.

(602) Defective Fermentations.

The success of fermentation is judged by the appearance of the yeast heads, a normal rate of attenuation, the formation of a good cover at the close of fermentation, with good break in the beer at the time of transfer to storage tanks. The appearance of the break must be judged in accordance with the flocculating character of the yeast. Fierce fermentation is undesirable, as it causes excessive precipitation of colloidal substances, with reduction in foam-holding capacity. A sluggish fermentation is equally undesirable and indicates a weak yeast or unsuitable wort composition. Yeast weakness may be revealed by failure of the low kräusen to cover the wort surface or defective attenuation which is not due to wort composition. Bladdery and boiling fermentations are liable to occur, as they do in top fermentations. Sometimes the head disappears and is replaced by local disengagement of gas, with bubble formation. Defective heads may in some cases be traced to low hop rates. This applies particularly to the final cover, which may be thin if the hop rate was too low. It is also ascribed, in other cases, to the use of over-modified or highly dried malt and even to bad ventilation of the fermenting cellar.

(603) Treatment of Yeast.

After the beer has been transferred to the storage tanks, the firm sediment of yeast is squeegeed into receivers placed below the fermenting vessels. Only the middle layer is used for pitching, those above and below it are contaminated with protein and resinous matter and liable to be more or less infected. The store yeast is stirred in water to break up the lumps and passed through a sieve to remove gross sedimentary matter. The dirty brown water is removed when the yeast has settled and is replaced by clean water. This is often carried out in wooden vessels, with several cork holes one above the other, the water being cooled with an ice swimmer or coil. Metal vessels, with double bottom for cooling, are used in new plants, such as that shown in Fig. 169. Yeast was formerly often washed by a continuous flow of water from below and over the top of the vessel, but this removes much of the lighter, more attenuative yeast. Care should be taken to prevent this by not washing too energetically or too long. The water used is always ice cold. Washing in pure cold water is held to improve the yeast. It becomes whiter, while particles of extraneous matter with very light dead cells, wild yeasts and bacteria are removed. Yeast is, however, weakened and the lag phase increased by excessive washing. The yeast can safely be stored for about a week under water at 32°–34° F., but should be pressed, if longer storage is required. It is then sometimes packed in airtight containers and stored at about 32° F.

STORAGE OF LAGER BEER

(604) Objects of Storage.

Bottom fermentation beer is usually lagered, or stored, for periods varying from 1 to 9 months, generally 6 to 8 weeks, after the completion of primary fermentation. Slow changes occur which may be comprised in the term maturation. These include :—

(1) Continuance of fermentation under conditions which will remove air and undesirable flavouring substances from the beer and ultimately lead to its saturation with CO_2.

(2) Promotion of changes in the colloidal constituents of the beer, resulting in aggregation and precipitation of proteins and in removal of hop resins under the influence of increasing acidity.

(3) Clarification, through deposition of yeast and suspended matter, together with the substances thrown out of solution.

(4) Improvement in flavour by production of minute quantities of esters, acids and other secondary products of fermentation.

Digestibility is held to be increased at the same time by changes in the protein and other beer constituents.

(605) Storage Operations.

Beer is usually transferred from the fermenting vessels to the storage tanks in Continental lager breweries at temperatures between 4° and 6° C. (39°–43° F.) and cooled gradually to between 2° and 0·5° C. (36°–33° F.) or, sometimes, to one or two degrees C. below the freezing point of water in the case of export beers. Rubber hose is widely used for running down the beer, but copper mains are used in some breweries. Sufficient healthy yeast is run down with the beer to re-start a slow fermentation, under the influence of agitation. The after-fermentation starts in the course of a day or two. A head forms at the surface of the beer and it was at one time customary to allow the yeast to purge into dish-shaped funnels inserted in the bungholes of wooden lager casks. This practice is discontinued in modern tanks. The cooling must be sufficiently slow to avoid abrupt check to yeast activity and permit of adequate fermentation and movement. It is usually due to heat exchange between the beer and refrigerated air of the cellar, through the material of the tanks, unless concrete storage tanks are used, when internal refrigeration is necessary.

It is common practice to divide beers from more than one brew over a number of tanks in order to equalise composition, but tanks should not be left incompletely filled for periods longer than a day. The low temperatures have a greater restrictive influence on culture yeasts than on some wild yeasts and delay in filling the tanks may encourage development of the latter, to the detriment of the flavour of the beer. The extra agitation secured by running down successive brews to mix in the tanks is advantageous when fermentations are inclined to be sluggish. It is also advisable to blend beers from different fermenting vessels of the same brew, when there is inequality in attenuations.

Wort taken from the fermenting vessels during the "low kräusen" period, containing actively fermenting yeast, is frequently added if the tank fermentation does not commence in a day or two, or when the beer is run down with very little yeast in suspension. It stimulates fermentation, but tends to reduce the stability of the beer, if added in excess. Quantities up to 5% should be sufficient. In some breweries yeast alone is added or pumping resorted to, if the fermentations in the tanks tend to be sluggish in starting.

(606) Storage Temperature and Time.

The temperature of storage has a great influence on the properties of beer. Clarification and maturation are more rapid at higher temperatures, but permanence of the brilliance secured by filtration is thereby diminished. Usual storage temperatures are between 0·5° and 2° C. (33° and 36° F.) but export beers are preferably stored at lower cellar temperatures and sometimes −2° or even −4° C. (28°–25° F.) is adopted. This entails longer storage to complete the separation of colloidal substances and make the beer as chill-proof as possible, without the use of any artificial aids, such as are described below. Export beers are frequently stored for 6 to 9 months, in place of the equal number of weeks more usual for beers intended for rapid consumption. The question of cost, with the delay in sales and utilisation of plant, have to be considered in connection with the storage period, which has also to be carefully controlled in relation to stocks, so that the beer can be racked or bottled when in the most perfect condition. Beers that are too young lack the fine aroma and palate typical of adequate maturation and they are also considered to be indigestible, but beer that has been stored too long becomes empty and lacking in flavour. The risk of deterioration through the slow development of the so-called sarcina, bacteria or wild yeasts, which may resist the low temperature better than culture yeasts, has also to be guarded against.

The amount and activity of the yeast present in the beer, with the composition and character of the latter, play an important part in governing the time of storage necessary for maturation. The stronger the beer and the less it has been attenuated in the primary fermentation the longer the storage required. Strongly hopped beers also require longer storage than others with less hops, while the storage may be materially reduced if the beer is required for rapid sale only. Pale beers are stored longer than dark beers of the same original gravity. Each type arrives at maturity, or the condition most applicable to its special character, in a different time. Thus if 10 weeks' storage were found applicable to a pale beer of moderate gravity, 7 to 8 weeks would probably be adequate for dark beers under similar conditions, while stronger beers might require 4–5 months. Very large vessels increase the time necessary for settlement, the slowness of clarification being particularly marked in large vertical tanks. The effect of surface on clarification has already been referred to, being delayed when the surface of the vessels is too glassy or smooth.

The fermentation during the first week or two of storage should be sufficiently active to result in brisk evolution of CO_2

and movement of the beer. A sluggish fermentation may be due to insufficient fermentable matter in the beer, or be caused by the use of either over- or under-modified malt and is particularly liable to occur when the malt has been overcured. The activity of the yeast is equally important, while the manner in which it separates has a great effect on the continuance of fermentation. A flocculating yeast may separate so rapidly, that attenuation ceases before it has reached the point necessary for stability. The greater attenuation required in the finer or export beers is secured by using powdery or non-flocculating yeasts. The beer should attenuate through about 4° of gravity (1% Plato) during the first four weeks and about 2° in the second month to bring its degree of apparent attenuation within 2 or 3% of the limiting attenuation.

(607) Bunging and Gas Saturation.

The saturation with CO_2 required to produce condition, with formation of a firm head and satisfactory foam-retention, was formerly secured by closing the lager cask with a wooden bung, when just the correct amount of fermentable matter was left to generate the desired pressure. The amount of pressure required varies with the composition of the beer and is regulated according to experience, between 0·25 and 0·35 atm. is usually sufficient. In modern tanks this is regulated by means described in Section 553, and still referred to as bunging apparatus. Closing the old wooden casks with a bung was a delicate matter. It might be done within the second week of storage, or not for a month and bending or bursting of the ends of the casks was to be feared, if the time was not well judged. The automatic regulators are simple in operation, can be set at any pressure desired and will keep the beer under that pressure when once it has been formed, so long as they do not become inoperative through accumulation of substances ejected with the foam, or, in some cases, get frozen. They act as safety valves and prevent damage to the tanks. As a rule they are set when the beer is run into the tanks and permit the CO_2 to escape freely during active fermentation, but retain it when the pressure settles down to the determined value.

The amount of CO_2 held in solution after storage at different pressures between 1° and 1·6° C. (33°–35° F.) is given in Table 230, after Schönfeld.

An increase of 1° C. in cellar temperature involves a decrease in the CO_2 content of the beer amounting to 0·01%. The formation of head and its retention depend on the composition of the beer as well as on the amount of gas dissolved. The storage pressure must consequently be regulated according to experience with the beer. For beers of 1048 specific gravity it is usually about 0·25–0·30 atm. (4 or 5 lb. per sq. inch). A pressure of 0·2 atm. is almost always insufficient, but over-bunging is liable to prove equally unsatisfactory, giving beer from which the CO_2 is given off as from soda water. Pressures of 0·4 atm. (6 lb. per sq. inch) are sometimes adopted for lighter beers. Variations in hydrostatic pressure cause an increase in the gas content of the beer in the lower part of tanks. At a depth of 3 feet the pressure is increased by about 0·1 atm. or 1·4 lb. per sq. inch, permitting a rise of 0·03% in gas content. About half of the CO_2 in finished beer is produced during storage.

TABLE 230.—BUNGING PRESSURE AND CO_2 SATURATION

Tank pressure gauge atmospheres	% CO_2 by weight in stored beer
0·30	0·390–0·410
0·25	0·375–0·395
0·20	0·360–0·380
0·10	0·330–0·340

The time during which the beer is kept under pressure has as much influence on the consistence and stability of the head as the pressure itself. During the early stages of storage, the gas comes off rapidly. The time of standing under pressure is measured from the time when a slight but constant rise is noted, with only slow bubbles of gas through the mercury column. At least a week is required for fixation of the gas after the maximum pressure is obtained.

(608) Maturation and Clarification.

Although lager beer is ultimately filtered before racking into casks or bottling, clarification is an essential function of storage, Rapidity and perfection of filtration, with economy in filtering material and labour, are in themselves important, but more particularly so because they are concomitant with quality in the finished beer. If clarification is inadequate, a more drastic filtration is required and this results in unnecessary and detrimental effects on palate fulness and on the properties of head-formation and retention. Sedimentation of yeast and other substances is intimately associated with maturation, but other factors also intervene in the development of the flavour and other properties associated with stored beer. Among these, the influence of saturation with CO_2 has already been dealt with. The changes which occur during storage are complex and the effects of many of the operating factors are still incompletely understood. Among these are the size and material of the storage vessels, the effect of their surfaces on sedimentation and CO_2 evolution, which act in a physical manner on the colloidal constituents of the beer. In addition, there are chemical changes in the composition of the beer during fermentation, including a gradual increase in acidity and formation of higher alcohols and esters. The temperature of storage and the time allotted to it, with the possible effects of enzyme action and of air, of organisms other than culture yeasts and varying racial characteristics of the latter, all have effects on clarification and through it on maturation.

Differences in the flocculating properties of yeasts affect clarification by variation in the rate of settlement but this does not imply that the influence of a flocculating yeast on the necessary separation of colloids will be as favourable as that of the more intensively fermenting non-flocculating races. Indeed, its effect is less, and non-flocculating yeasts are to be preferred for the permanent brilliance required in export beers. This depends on changes in the physical condition and state of dispersion of colloid constituents of the beer, derived mainly from the proteins of the malt and resins of the hops. These changes lead to gradual precipitation and adsorption of colloids on the surfaces of the yeast and tank, which are favourably influenced by the motion produced by continued fermentation and by its effects on the composition and reaction of the beer. Comparatively slow yeast settlement may generally be regarded as favourable to beer quality, so long as it is not accompanied or caused by the presence of wild yeasts, " sarcina " or other infecting organisms. Stability, in the sense of persistent brilliance after pasteurisation and absence of liability to chill haze, can only be secured by long maturation, accompanied by full attenuation. More rapidly flocculating yeasts are suitable for draught beers for which adequate maturation, stability and gas saturation can be secured more quickly.

(609) Flavour and Aroma.

Flavour and aroma are greatly influenced by storage and are important features in maturation. The removal of flavours typical of young beer is emphasised in connection with the

Nathan system of fermentation and is effected in the usual storage tanks by prolonged evolution of CO_2. In addition there are gradual changes in the nature of the hop bitter substances, dependent to a considerable extent on the development of acidity and precipitation of colloidal substances. These result in the gradual elimination of harshness and production of a milder and rounder flavoured beer. Though by-products are formed in the primary fermentation, changes in flavour which can only be attributed to the slow production of esters and acids also occur during storage and, with the gradual decrease in fermentable substances and increase in alcohol content, finally give the flavour of stored beer. The progress of maturation is judged by examination of samples taken from the appropriate tap. Attention is given to the manner in which the head is formed or broken up when the glass is rotated, to its quantity and persistence. The flavour is also noted and the readiness for filtration, judged by the brightness of the beer and the nature of the break.

(610) Chips.

Fermentation can be materially accelerated in tanks by the use of hazel or beech wood strips, usually referred to as chips. They are generally about 15 to 20 inches long by 1 to 2 inches wide and their beneficial influence may be attributed largely to surface effects. They serve for collection of yeast and separated colloidal substances and also facilitate the discharge of CO_2 bubbles. They are comparatively ineffective when fermentation is sluggish, in which event the beer should be kräusened as well. They must be boiled and washed previous to use, to avoid tainting the flavour of the beer, and thoroughly sterilised after use by boiling and washing in running water before they are used again. The great objection to their employment and the reasons for which they have come into disrepute arise from the risk of infection by organisms held in cracks and fissures, and from the waste resulting from absorption of beer. These risks have been minimised by the use of wooden chips covered with pitch and of aluminium strips, foil and rings, with the result that renewed attention has been given to their use, particularly in large tanks. The surface of the aluminium is suitably roughened, embossed or pierced to make its effects approach as nearly as possible to that of wood and, in many cases, its lightness permits floating and circulation in the beer, when buoyed up by gas bubbles, but it is doubtful if these devices are as efficient as wooden chips.

Seibel suggested the use of rolls of aluminium foil, about 0·01–0·02 mm. thick and coated with pitch. They are used at the rate of about 2 kgm. per hecto of beer, giving a total surface of about 200 square metres. Tanks have also been fitted with suitably constructed aluminium devices with the object of increasing the area of deposition. Powdered and sterilised wood, known as Bio-chips, is very effective and largely used. It is impregnated with Mammut and supplied in closed packages holding the required quantity for a tank, to which it may be added without fear of infection.

(611) American Storage Processes.

Modifications of the storage process described have been developed in America. These have been made in view of the wide adoption of carbonation in place of complete natural conditioning. The excise requirements of a Government Tank and use of beer meters also introduce variations in procedure. In some cases there is no after-fermentation during the storage. The beer, fermented almost to the limiting attenuation, and cooled very fully in the fermenting vessels, is run through a carbonating pump and counter-current cooler to reduce the temperature to 32° F., and stored in tanks at that temperature for one to two months. In some breweries, the yeast is removed by passing the beer through a Kieselguhr filter on its way to the storage tanks. The low temperature of storage facilitates clarification of the beer during this period of maturation.

The beer may be conditioned by kräusening in the tanks, by carbonation or by combination of the two processes. Kräusening in " Chip " tanks adds a certain amount of hop resins and wort sugars, which may not be completely fermented in the process, with the result that the beer produced is both sweeter and more bitter than that which is carbonated. Its stability is also less. The quantity of kräusen added varies considerably and may be 15 or 18% for draught beers, but only 5 or 10% for beers of greater stability. Chips are very generally added to facilitate clarification and pressures are allowed to rise to about 5 lb. A lower pressure, about 2 lb., followed by carbonation gives good results. Kräusening is much less general than formerly and the majority of brewers rely entirely on carbonation.

A very complete installation for carbonating with fermentation gas is illustrated by Figs. 176 and 177. The gas-collecting compressor is shown in the centre of Fig. 176, cylinders for storage of the CO_2 under a pressure of 250 lb. per square inch on the left of Fig. 177 and Taber carbonating pumps on the right. These centrifugal pumps take beer from the fermenting vessels. CO_2 is injected in the delivery main above the pump, at a pressure of

30 lb., and is absorbed as the beer passes through a counter-current chiller to the storage tank against a back pressure of air at 15 lb. The tanks are fitted with a low pressure relief valve, through which the counter-pressure is relieved as the beer rises in them. The beer is then stored for a month or two at 32° F. for stabilisation by precipitation of haze-forming protein complexes. (Section 709.)

American lager beer may be stored at atmospheric pressure or at any greater pressure up to the degree of CO_2 saturation required at racking. They should be subjected to a slight " primary " carbonation, even when stored under atmospheric pressure, to ensure that the beer, after chilling to 32° F., is saturated with CO_2 and that the space above it in the storage tank is full of gas, in order that absorption of air may be avoided. Alternatively, the primary carbonation may be against a counter-pressure of 2–5 lb. or the beer may be fully carbonated against a pressure of 10 15 lb.

(612) Bulk Carbonated Ales.

Similar methods of carbonation are applied to top fermentation beers in America and, to a comparatively small extent, in this country. They are usually conditioned in tanks at about 60° F. in the manner described in the Sections on Bottling. The pressure may rise to 25 lb. in 6 or 7 days and is not allowed to rise higher, the tanks being vented daily to prevent this during further storage, which may extend for another 10 days. The beer is then passed by an air top pressure of 25 lb. through a chiller-carbonator into a storage tank against a back pressure of 7–10 lb. It is stored in these tanks at 32° F. for 10 days, a fortnight or until required for racking. A plate-exchange chiller (Section 517) serves admirably for bringing the temperature to 32° F. and carbonating by CO_2 injection at 58 lb. on the warm side.

Bulk transport has provided a successful complement to tank conditioning and filtration where the trade permits of two deliveries a week. Insulated road tanks, divided in 2 or 3 compartments if necessary, and mounted on lorries are filled against a CO_2 pressure of 5 lb. From these the beer is delivered through a hose to 2- or 3-barrel tanks in the houses. A recently patented development by Worssam and Fox provides for delivery of naturally conditioned beer through a chiller, pump, centrifuge and meter to the tank wagon, and thence to the small tanks in a thermostatically controlled cellar. A CO_2 cylinder, mounted on the wagon, supplies pressure for the tanks and charges a gas reservoir in the cellar for beer raising. The beer, on its way to the bar, is warmed to the most acceptable temperature by passage through a thermostatically controlled water bath and lightly filtered for delivery in a brilliant condition.

(613) Summary.

Bottom fermentation processes, used for lager beer, differ from top fermentation, adopted for ales, in that the temperature ranges between 41° and 56° F., generally between 42° and 49° F. in continental practice, while the yeast settles at the bottom of the fermenting vessel at the close of fermentation, instead of rising to the surface of the wort. Fermentation is considerably slower, as a result of the low temperature, than in top fermentation systems. Lengthy storage of the beer between the primary fermentation and racking is normally essential, and gives the name lager or "stored" to the beer. Fermentation in the storage tanks is due to primary yeast, carried down with the beer from the fermenting vessel. The CO_2 evolved is at first allowed to escape, carrying air and unpleasant, volatile flavouring matters with it. During this earlier stage of storage, the beer is gradually cooled to about 36°–33° F. or lower for export beers. After about a fortnight the storage tanks are "bunged" and the pressure allowed to rise to 4 or 5 lb. per square inch, at which it is maintained during the maturation and clarification period of 6 weeks to 3 months. Long storage is avoided in the Nathan process by washing out the "young bouquet" by a stream of CO_2 after the primary fermentation is completed. The beer is then cooled to 32° F. and saturated with CO_2. American lager beers are usually fermented almost to the limit and carbonated, either before or after storage. In some cases conditioning is obtained by kräusening, or addition of fermenting wort to the storage tanks. Carbonation may be completed with fermentation or tube gas before racking or bottling. Top fermentation beers are carbonated in bulk by similar methods, but it is usual to submit them to a preliminary period of conditioning in tanks at about 60° F.

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2. R. Lieske and E. Hofmann, *Biochem. Z.*, 1929, **219**, 448 ; *J. Inst. Brew.*, 1930, **36**, 199.
3. F. Windisch, *Woch. Brau.*, 1930, **47**, 82 ; *J. Inst. Brew.*, 1930, **36**, 199.
4. F. Stockhausen and F. Windisch, *Woch. Brau.*, 1928, **45**, 277 ; *J. Inst. Brew.*, 1928, **34**, 501.

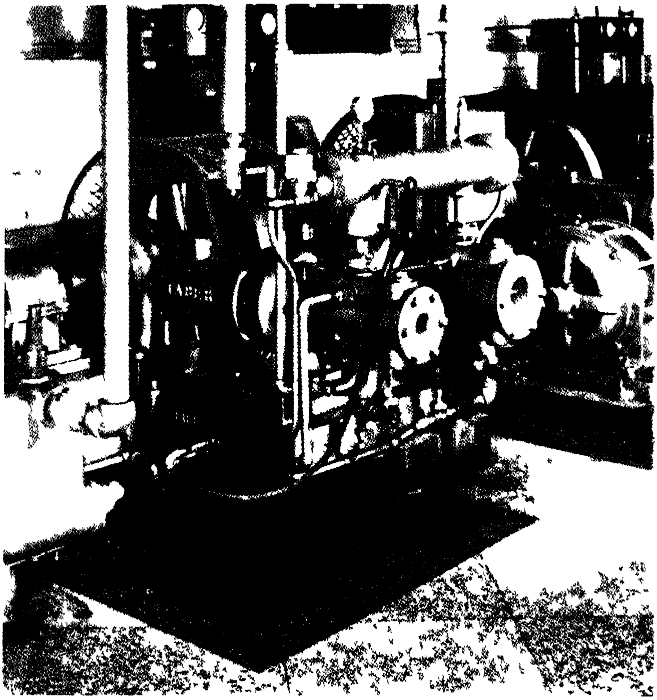

FIG. 176

[illegible] COMPRESSOR

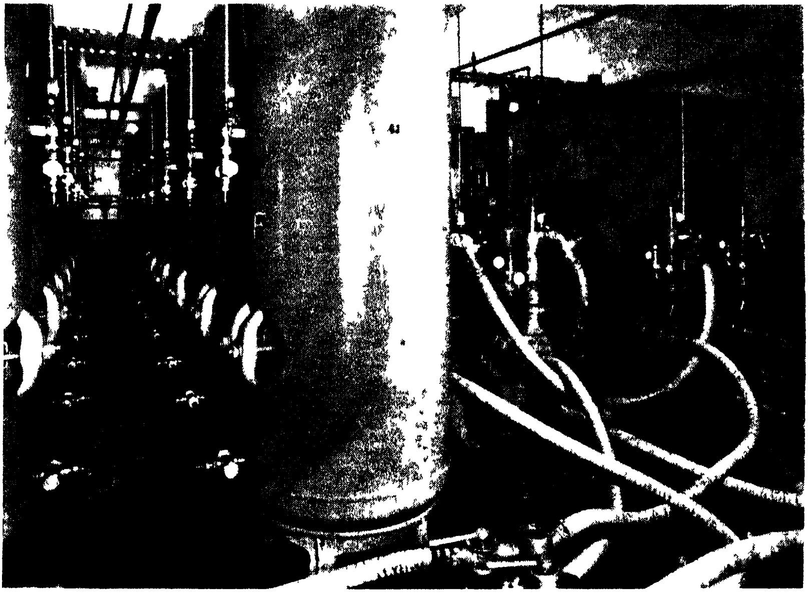

FIG. 177

CO_2 STORAGE TANKS AND CARBONATING TUBES

CARBONATION WITH FERMENTATION GAS

(BREWING CORPORATION OF AMERICA, CLEVELAND, OHIO)

Facing page 850

RACKING
AND
CELLAR MANAGEMENT

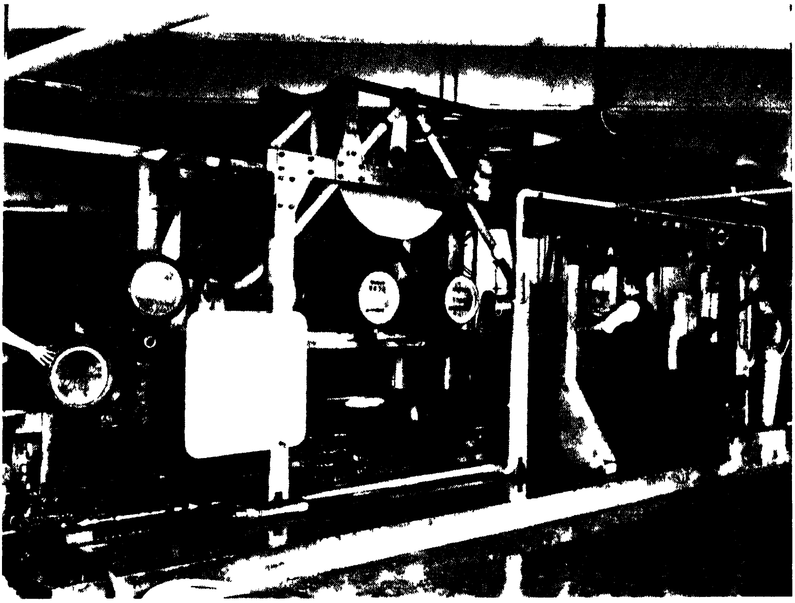

FIG. 178

AUTOMATIC CASK WASHER

(THE [illegible] BREWERY [illegible])

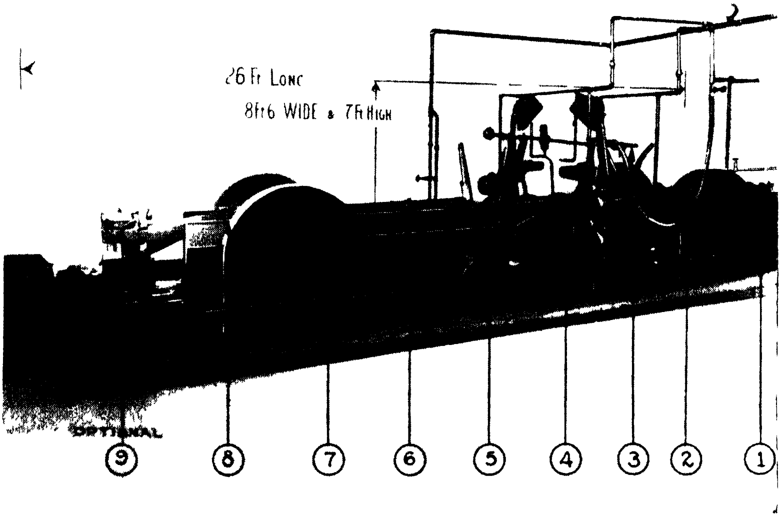

FIG. 179

AUTOMATIC WASHER FOR PITCHED CASKS

Facing page 851

CHAPTER XXXV

RACKING AND CELLAR MANAGEMENT

CASKS

(614) Treatment of Casks.

Oak has proved the most suitable timber for casks. Many others have been tried, but most have failed on account of cost or some characteristic of texture or flavour which renders them unsuitable for cask making or beer storage. The so-called Crown Memel staves, formerly obtained from North Russia, but recently from Poland, are the favourite material in this country. Good American white oak appears to be equally satisfactory, is tougher and more elastic than Russian or Polish oak, but some darker or reddish timbers give a flavour to beer, even when only one stave has been used for replacement. Casks must in all cases be "seasoned" before use. This process usually consists in filling them with boiling liquor, in which soda or common salt is dissolved. A gallon measure of the latter per barrel, for example. The casks are drained after 12 to24 hours' treatment and thoroughly steamed. After which they may again be filled with hot liquor or washed in the usual way. It is advisable to fill them first with a quick sale stout or mild ale.

Surplus casks can be stored either in the open or under cover, preferably in a cool cellar. Closed casks stored in the open must be hosed frequently in hot weather to prevent shrinkage and ingress of rain through opened staves, which causes them to become stinkers. It is preferable to unhead, scrub and up-end them to dry. After making sure that they are sound, the hoops are driven, and the casks internally brushed with bisulphite of lime. They may then be piled, with the bungholes down and heads placed inside. Casks stored under cover are not unheaded. They are washed out with bisulphite of lime, drained and shived.

It is necessary to allow reasonable overmeasure in cask capacity for the unsaleable bottoms, but the actual allowance varies with the conditions of sale. Half a gallon per 36 gallons is quite usual and new barrels are commonly made with a capacity of 37 gallons. An excessive allowance materially raises the apparent racking loss. Gauging is carried out by filling with water from a measured tank or by weighing. The latter process is more accurate, and more rapid when many casks have to be dealt with. The cask is tared on a steelyard or platform machine, filled with water and again weighed. Each cask is marked with its capacity in countries where they are used as beer measures.

(615) Defective Casks.

Infection from casks is a not infrequent cause of returned beer, but elimination of the danger may be costly and involve drastic treatment, which reduces the life of the casks. Micro-organisms penetrate some distance into wood. Bacteria may be be found a quarter of an inch below the surface of comparatively new casks, and it is difficult to effect anything more than surface sterilisation with boiling liquor and steam, unless these are applied for quite lengthy and generally impracticable periods. Briant[1] found that the temperature of the wood, one-eighth of an inch from the surface, was only raised to 120° F. by 10 minutes' steaming. The danger is greatly reduced in rapid trade, but customers must be impressed with the damage which results from failure to cork empty casks. Casks returned from trade should also be washed without delay. It is far from possible to make sure that casks are clean from sight and smell alone. Tests should frequently be made by swilling them with about a pint of sterile water and plating about 0·5 ml. on wort gelatin or agar, if the appearance of the water, as so often happens, is not so bad as to make a bacteriological test quite unnecessary.

Among the obvious defects produced by the development of bacteria and moulds on the surface or in the pores of the timber are "acidity," "mustiness" and "fustiness." In some cases the odour is described as "casky," and in bad cases, through access of rain water, the casks become "stinkers." Acid and slightly musty casks can be cured in many cases by soaking with boiling liquor containing soda, followed by steaming, before they are washed. Worse cases must be shaved or hollowed and fired before they are washed, but there appears to be no cure for a badly fusty cask. Many "foul" casks can be saved by unheading and scrubbing, followed by "dressing clean" and firing, after which they are steamed for at least three hours.

(616) Metal and Lined Casks.

Despite the general suitability of wood for beer containers, the drawback of porosity has led to the introduction of metal casks and various types of lining. Suitable metals are unobjectionable from the hygienic point of view, but restrictions on cost and weight have made it difficult to construct a metal container which would withstand shock without deformation, and be suitable for transport. Stainless steel is the best material. Various types are available, constructed in a form to withstand shock and surrounded by an outer shell of steel or wood to overcome the drawback of rapid heat transfer. Steel casks with burnt-in enamel linings are also available, while aluminium is used for some purposes, though delicate and readily corroded by atmospheric influences. Metal casks are particularly useful for carbonated beers, avoiding the loss of gas which occurs in unlined wood. They are also very convenient for bulk pasteurised beers. Special bushes are fitted and extractors supplied for withdrawing the beer without loss of gas.

Various types of elastic linings which sink into the pores of the wood and firmly adhere to it, giving a hard and smooth surface, have, up to the present however, provided the most generally acceptable alternative to plain wooden casks. Brewers' pitch has been used for many years for lager casks and is useful for other filtered beers. It prevents contact with the wood and escape of CO_2, but some types soften at rather low temperatures, so that the hops and deposit in cask-conditioned beers adhere to the lining in the summer, and are difficult to remove on washing machines. Brewers' pitch is compounded in various ways from the resin of coniferous trees, which is melted and boiled to drive off volatile constituents. The brown residue is usually known as colophony. This is melted and mixed with resin oil, linseed or cotton-seed oil and, sometimes, with paraffin wax or ceresine to reduce the viscosity and make thinner coatings possible. The pitch should not contain more than 0·2% of inorganic matter, and be free from water. Its melting point should be about 113° F. If it is higher, the melted pitch flows with difficulty, solidifies too quickly and does not penetrate the wood. A lower melting point means that the pitch will soften and melt in the sun. It should not soften below 80° F. and harder pitch, softening at about 100° F., is to be preferred. The melting point of new pitch may be about 107° F., but this rises to about 120° after heating for some hours and evaporation of volatile constituents. Rather higher melting points are preferable for cask-conditioned beers, to reduce the tendency to adhesion of hops and sediment. A good pitch should communicate little, if any, flavour to beer.

Certain proprietary enamels, which sink well into the wood and give a harder surface, are preferred by many brewers and have come into extensive use. They are easily applied to old or new casks, should give no flavour to beer, and remain unsoftened if the casks are exposed to the sun. Paraffin wax is less commonly used, as it is liable to soften in the summer unless it has a melting point of at least 120° F. Whatever the nature of the lining, it must resist the shocks inevitable in transport and neither flake nor crack.

Among the advantages of linings are protection against flavour from the wood, and the possibility of bringing tainted casks into use. They prevent much of the damage which occurs when wooden casks are left uncorked, greatly facilitate washing, and save cost in steam and boiling liquor. It must not be thought that the danger of cask infection is eliminated by their use, though it is greatly reduced. The high temperature attained during pitching renders the casks nearly sterile, but they do not remain so during storage for a few days. This difficulty has been overcome (Mendlik[2]) by pouring 1 ml. of a saturated solution of SO_2, about 6% concentration, into the newly pitched casks and closing with a rubber bung. The slow diffusion obtained in this way is more effective than a short treatment with a higher gas concentration. The casks are swilled with liquor to remove SO_2 immediately before filling.

(617) Lining Casks.

New casks should be seasoned before enamelling, by filling with boiling liquor and soaking for 12 hours. After this they are emptied, steamed, and thoroughly dried to a depth of one-eighth of an inch at least. Old casks, which are tainted, may have to be steamed for 20 minutes or more. Stinkers should be shaved and fired before steaming. Two or three coats of enamel can be applied to the dry timber with a brush, after the cask has been warmed by fire, but it is generally preferable to use a spraying machine for enamels, as well as for pitch. The solvents, of which trichlorethylene is an example, are unpleasant to deal with. Two or three days are allowed for enamel to harden, after which the cask is filled with very hot liquor and allowed to stand for 24 hours, and again filled for 2 hours to remove all flavour. The ordinary cask-washing process can be followed subsequently, though it is desirable to use a perforated, rather than a slotted, nozzle to prevent stripping of pieces of enamel. Linings of this type should remain in good condition for a year or more, but must be renewed if parts wear off. A lined cask which has become a stinker is unheaded, scrubbed with boiling liquor, dried and relined.

Simple pitching machines consist of a cauldron, in which the pitch is melted over a coke fire or by gas, and forced by compressed air through a spraying nozzle into the cask, from which the excess of new and melted old pitch flows back into the cauldron. New pitch becomes sufficiently fluid to spray at 300°–320° F. but temperatures between 430° and 490° F. become necessary as volatile constituents are driven off and returned old pitch accumulates in the boiler. Resin oil is added to the hot pitch, to replace that lost and keep down the working temperature as old pitch accumulates in the mixture. The casks are generally depitched and repitched over different nozzles in newer machines, the recovered pitch being regenerated by mixing with the necessary quantity of oil. In some automatic machines, the casks are first dried with hot air at 230°–265° F. and then depitched by injection of used pitch, the whole running back into the boiler. The cask then passes over another jet and is sprayed with new pitch from a second boiler at about 300° F., rising to 350° as the heated pitch becomes more viscous. Hot air is then injected to remove drops of oil and inflammable vapours, which are led away through a chimney to avoid the danger of fire. The casks are finally rotated on a series of rollers to spread the pitch evenly. A hectolitre cask may require 0·35–0·7 kgm. of pitch. In many breweries the casks are repitched every time before use. In others this is only done every two or three fillings, when they are marked with variously coloured paints to indicate the number of times they have been in trade. The hoops are automatically driven, and the casks tested for leakage by a machine which lowers them into water, while under an internal air pressure of two atmospheres.

(618) Cask Washing.

More or less automatic machines have practically displaced the laborious process of unheading and scrubbing, but this still remains advisable on occasion. The old process of " running in " or filling previously swilled casks with boiling liquor, which is left over night to exert its full action, or the alternative of about three-quarters filling the cask with hot liquor, which is then boiled by steam injection, are very effective, though costly and somewhat detrimental to the casks. These methods, followed by a thorough rinse on a jet and further steam treatment, are difficult to improve upon, unless it is by the use of a " rumbler." This is a machine on which the casks, containing about a gallon of gravel and water, are turned over and over for a lengthy period and thoroughly scoured. It, or some similar scouring process, is in many cases essential to remove a hard yeast deposit and secure a really clean cask. Unfortunately a choice often has to be made between some drastic process, involving increased cost and wear of the casks, and more rapid methods which may occasionally lead to disappointment and early frets in the beer.

Automatic machines must be carefully watched to avoid reduction in liquor temperature, inefficient or partly clogged jets, inadequate pressure or insufficient time of action. No machine can be considered entirely satisfactory, unless it thoroughly scours the interior of the cask with boiling liquor or steam but, with ordinary attention, the various types available deal efficiently with most casks. Reliance has to be placed on smelling and inspection with an electric lamp or acetylene flare to pick out the few that require a second passage over the jets or special treatment. Many consider it necessary to remove the hops and bottoms with cold liquor before the casks are submitted to the hot spray, which tends to cake sediment on to the wood. The preliminary wash is, however, omitted with some machines, with generally quite satisfactory results.

With one type of washer, the cask is centred over a jet on a turntable, caused to revolve by pulling a lever, which at the same time admits a spray of boiling liquor, at a pressure of 200 lb. per square inch. This is followed by a blast of compressed air to discharge the water, spent hops and bottoms. One man can deal with 60 casks an hour with this machine, given the rather short washing time of 30 seconds, or with rather less than twice as many on two nozzles. In some machines the jets revolve, while the casks are stationary. Others consist of one or a number of jets connected to a tank of boiling liquor. A three-way valve first admits hot liquor to swill out the cask. This is followed by steam, while the third position of the valve admits liquor to the boiler, to be heated while the cask is removed and replaced by another. Automatic machines, with a number of jets and a set of external brushes, such as the Goliath shown in Fig. 178, are also extensively used in large breweries. The cask is first placed in position with its bunghole over a stop, and is then mechanically lifted over a succession of jets. The first jet in the machine shown delivers a large volume of cold liquor to swill out the cask. This is followed by six jets delivering liquor at about 190° F. and two steam jets. The hot liquor jets have a fine slot to produce a cutting action on the sediment in the cask. The valves are operated by the weight of the casks. Centrifugal pumps supply the liquor jets at a pressure of about 60 lb. per square inch. The quantity of liquor required is very considerable, about 3½ gallons for a 9 seconds' treatment per jet, but this is usually made up from the warm outflow liquor from attemperators and refrigerators. The brushes are placed half way along the machine, the casks being revolved between them and again automatically centred before passing to the next jet.

Enamelled casks can, in many cases, be washed in the same way, but the glossy surface makes it unnecessary to employ boiling liquor to remove the deposits, thus saving boiler costs, but risking infection. Boiling liquor cannot be employed for washing pitched casks. An American automatic machine, the 1939 model of the Schock automatic washer, is shown in Fig. 179. Liquor at 120°–130° F. is used. The casks are fed automatically to (1), from which they are carried to a rest (2), provided with a spray nozzle to remove labels. After this the casks are turned by revolving tractor wheels to bring the bunghole upwards at (3) where they are partly filled with warm water and externally sprayed. The casks are then oscillated on rubber-covered eccentric traction wheels beneath four brushes, while they are externally sprayed. They are then turned by revolving wheels and come to rest with bunghole down to drain. At (6) and (7) the casks are placed over hot water jets, revolving counter-clockwise and clockwise, respectively. A cold water spray is applied at (8), after which the casks are carried directly to the discharge skid or, with the bunghole down for draining, to a discharge station (9), arranged to deliver either to the right or left. Automatic machines are designed to take a wide range of cask sizes.

Casks must in all cases be allowed to cool before use. In lager breweries this is generally effected by rolling them to the cold racking cellar, where they remain over night. A refinement has been adopted in a London brewery, by which the casks are treated with a current of refrigerated, filtered air, in order to cool them rapidly, and simultaneously eliminate the danger of aerial infection. It is, however, more economical to cool the beer sufficiently to allow for a rise of two or three degrees of temperature after racking into normally cooled casks.

RACKING

(619) The Beer at Racking.

It is very desirable that beers should be sufficiently cooled to allow for the inevitable rise of a degree or two at racking, and further rise during subsequent storage or transport in warm weather, so that they may be served at the most acceptable temperature, between 55° F. and 58° F. for draught ales. It is equally important to avoid a fall of temperature after fining, which leads to greyness in cold weather. It may sometimes be advisable in the summer to reduce the temperature of the beer well below 53° or 54° F., suggested as racking temperature in Section 580. This can be effected by chilled liquor or brine in a plate-exchange or counter-current refrigerator between the fermenting vessel or settling back and racking machine. It should be assisted, whenever necessary, by cooling the air of the cellar in which the beer is to be stored for a few days to about 55° F. This is among the most useful applications of air conditioning, and goes far to eliminate the danger of frets. The racking room or cellar should also be cooled to a temperature corresponding with that of the beer.

The sediment in a newly racked beer should not show any wild yeasts when microscopically examined. Their presence is an indication of abnormal infection. They may, however, be present in a form or quantity which escapes detection, or infection may be derived from the casks. In such circumstances they are capable, under favourable conditions, of development and may produce a fret some few days after racking or when the beer is in the customer's cellar. Wild yeast will not develop to any marked extent when there is a sufficient quantity of primary yeast to promote a natural cask condition, with or without the addition of primings. Yeast is in a dormant condition after settling in the fermenting vessels, but is activated by aeration of the beer at racking. In some cases flagging primary yeast can be brought into active condition by priming and rolling but, if it has lost its vitality, rolling may be harmful by encouraging wild yeast. These are activated, at least equally with primary yeast, by oxygen and movement in the beer, but cannot become dominant if the oxygen if rapidly absorbed by the much greater quantity of competing culture yeast. Cask-conditioned beers must, consequently, not be too bright at racking but they must, on the other hand, not contain so much yeast as to produce tumultuous condition. It is necessary to distinguish turbidity due to yeast from that caused by an excessive quantity of amorphous matter resulting from defective flocculation and sedimentation in the copper or during cooling, which is always detrimental.

The quantity of yeast required in suspension at racking to produce satisfactory condition in cask or tank varies inversely with the amount of residual fermentable matter. Rather more should be allowed with a fully attenuated beer. Burns[3] gave the following quantities for one particular top fermentation brewery,

0·1–1 million cells per ml. was the optimum.

1–5 million cells was not likely to cause difficulties.

Over 5 million cells was liable to produce a primary yeast fret in the casks with fining difficulties.

In some cases it may be necessary to add fresh yeast with the primings, in order to prevent the lack of condition that results from racking too clean and may lead to wild yeast frets and fining troubles. 3 lb. of pressed yeast per 100 barrels of beer is in many cases adequate for this purpose. It means an approximate addition of 0·4–0·5 million cells per ml.

(620) Measurement of Yeast in Suspension at Racking.

The importance of regularity in cask condition makes it very desirable to institute some method of control. For this purpose, the number of yeast cells in unit volume can be counted with a microscope and haemocytometer, but this is a very tedious proceeding. In some breweries a sample of beer is centrifuged in a specially graduated tube and the volume of yeast read off. This method is rapid, but may be vitiated by the formation of a bubble of CO_2 in the yeast deposit and, on the whole, turbidometric measurements are to be preferred. Photo-electric apparatus or a Nephelometer are used for this purpose, but the necessary apparatus is rarely to be found in smaller breweries and some simple but reasonably reliable method must be used instead. In some cases, this resolves itself into visual estimation of the quantity of deposit in a sample of the beer taken the evening before racking and set up in a conical glass. This gives a very useful indication of the regularity of settling or of variations in the quantity of yeast.

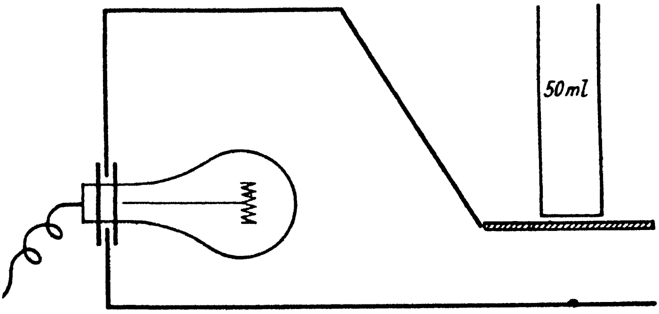

FIG. 180

APPARATUS FOR ESTIMATING THE NUMBER OF YEAST CELLS IN BEER (J. A. BURNS)

Burns described a more accurate method in which the turbidity of the sample is compared with that produced by suspension of known quantities of yeast in equal depths of beer in Nessler tubes, by means of the apparatus shown in Fig. 180. A 5%

pressed yeast suspension was found to contain 240 million cells per ml. 2·1 ml. of this, diluted to 100 ml., gives a suspension of 5 million cells per ml. 1, 2 and 3 ml. of the latter suspension are measured into three Nessler tubes containing 5 ml. of filtered beer of the same colour as that to be tested. The volumes are then made up to 50 ml. with acidified tap water. 5 ml. of the beer to be tested is placed in a similar tube and diluted to 50 ml. with acidified tap water, any froth formed being broken by touching it with amyl alcohol. The Nessler tubes must be chosen so that the graduation marks are all at the same height.

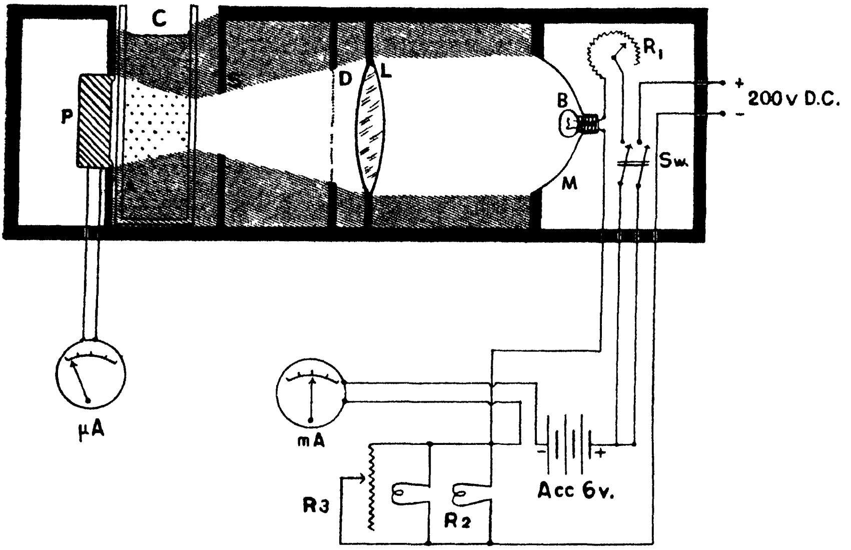

FIG. 181

PHOTOELECTRIC TURBIDIMETER
(THORNE AND BISHOP)

They are placed on the glass plate of the box, shown in section in the figure, immediately over a pencil line on a sheet of white paper, illuminated as indicated. This is viewed vertically downwards through the tube of beer, the turbidity of which is assessed by comparison with the visibility of the line through the standard tubes. If the yeast count is over 3 millions per ml. the pencil line is not discernible and the sample must be diluted with a proportion of filtered beer.

Thorne and Bishop[4] described a method of photoelectric measurement by which the proportion of light stopped by a yeast suspension could be correlated with its dry weight or cell number. The apparatus is shown in Fig. 181. The optical parts are enclosed in a light-tight wooden box, painted dull black within. The light source, B, is a 6v., 6w. electric lamp mounted in a parabolic mirror, M, giving a parallel beam of light, which is rendered structureless by means of the diffusing screen, D (a sheet of ground glass adjusted to the required degree of translucency by coating with a film of transparent cellulose lacquer). The beam of diffused light falls on the yeast suspension in the glass cell, C ; the light emerging from the latter falls on the photoelectric cell, P, in which it induces an E.M.F. the magnitude of which is measured by the microammeter, μA. The photoelectric cell used is the " Photronic " of the Western Electrical Instrument Corporation. In conjunction with it a 0–250 microammeter is used, the current recorded varying practically linearly with the intensity of illumination on the cell. The glass cell, which may be a flat-sided bottle of 3 mm. internal diameter, containing the yeast suspension, is held between the two stops, S, which prevent entry of stray light into the Photronic cell and ensure that the surface of the suspension shall introduce no internally reflected light into it. The intensity of the incident light may be adjusted with the 2-ohm variable resistance, R_1. A convenient way to achieve a steady intensity of illumination is to have the 6v. accumulator, which discharges through the lamp, B, continually recharging from the mains (D.C., or A.C. rectified) with the help of an appropriate lamp resistance board, R_2, and variable resistance R_3. mA is a milliameter to balance accumulator output and mains input, Sw a double pole switch for simultaneous control of mains and accumulator circuits.

The lamp is switched on and adjusted with the resistance R_1, to give a deflection of approximately 250 on the microammeter, when the cell, C, contains only water. The water is replaced by the well-shaken yeast suspension and the new microammeter reading noted. The current set up by the photoelectric cell varies approximately inversely with the concentration, P, of the yeast suspension, over the range of concentrations encountered in practice. P can itself be used as a measure of yeast quantity. Its value is independent of the colour of the beer and nearly independent of turbidity arising from other causes. A correction for turbidity can be made by taking a microammeter reading of the wort or beer, after filtering off the yeast.

The yeast concentration at racking can also be standardised against nephelometer readings with the Pulfrich Photometer, which vary almost linearly with the dry weight over a range of yeast concentration of 0–0·15 gram per litre. The beer, in a beaker, 35 mm. in diameter, is placed in the chamber containing distilled water, while a parallel beam of light from the lamp passes through it. Some of the light is scattered by the yeast and that part which is scattered through an angle of 45° enters one limb of the photometer. At the same time part of the light from the lamp is reflected from a glass plate and illuminates an opal disc, from which a constant proportion of the light is dispersed at 45° through the other limb of the photometer. The standard disc of brightness 4 is used. The drumheads, graduated in percentages, operate a diaphragm, so that the light in the two limbs can be adjusted to equal brightness in a half-shadow eyepiece, after passing a monochromatic filter. The results are expressed in terms of the body of standard turbidity provided. The monochromatic filter used is the green filter "L11." The nephelometer reading of the turbid beer is corrected by subtracting from it the reading after filtration. Over the range of yeast concentrations of 0–0·15 gram per litre, the dry weight can be estimated from the equation

$$D = 0{\cdot}00027\ T$$

in which T is the drum reading, or the moist weight by multiplying the above result by 5. Allowance must also be made for the degree of dilution.

(621) Racking Top Fermentation Beers.

Uncarbonated top fermentation beers, which include the bulk of British draught ales, are racked either directly from the fermenting vessel or from a racking or settling back, to which they are run down from the fermenting vessels. This may serve only as a reservoir, situated in a convenient position for cask filling, or may provide a means for further clarification of the beer by sedimentation during 2 to 12 hours. The racking back is also frequently used for the addition of primings, colouring and, sometimes, finings, but many prefer to add these to the individual casks. It has the disadvantage that the beer loses CO_2 and is aerated. It also means another vessel to clean and an additional possible source of infection. The balance between advantage and disadvantage may depend on the type of yeast, and the effects of transfer on regularity in the yeast content of the racked beer. Absolute regularity can never be attained, but very little of a firm sediment should be carried forward to the casks, if the thimble of the racking main is properly adjusted. A yeast which settles down firmly on the bottom of the fermenting vessel, yields beer which can be quite well dealt with without a settling back, while the latter can be advantageously used with the slower settling powder yeasts. The period of rest in the settling back must be judged from the quantity of yeast in suspension and the rate at which it settles.

The usual alternative to a settling back is a racking cylinder, such as that shown in Fig. 183. It should be of sufficient size to ensure even flow of beer to the number of filling heads employed. Beer flows gently to it from the fermenting vessel, the level being maintained at about half the diameter of the cylinder by means of a float valve. It is provided with a swinging door at one end, locked by wing nuts, so that it may be readily cleaned. The racking heads, connected by rubber hose to the bottom of the cylinder or close to the bottom of the racking tank, as the case may be, carry copper tubes of slightly smaller diameter than the bunghole of the casks, and sufficiently long to reach nearly to the bottom of the latter. They are fitted with an expanding rubber joint to make airtight closure with the bunghole when the beer valve is operated, after lowering the tube into the cask. They are also provided with a beer return tube and sight valve. A certain amount of frothing and overflow always occurs, but this beer should not be returned to the racking tank, as it may carry infecting material from the casks. It should be carried to an overflow tank for pasteurisation or filtration before blending, or to a small cylinder, from which it is returned directly to the casks. Cylinders serving three casks are shown in Fig. 182. The filling tubes and head are counterpoised, so that they may be lowered into the casks without effort. They can also be easily dismantled for cleaning.

The required quantities of primings and colouring are placed in each cask before they are filled, unless they have been previously added in bulk or are to be added immediately before dispatch. Dry hops are also added. The casks are rolled under the filling heads and, after filling, are closed with a shive or, in some cases, with the convenient, but unhygienic, alternative of a bung and square of sterilised hop sacking. They are then rolled away to the cellar for after-treatment and storage or direct to the loading floor. The wear of the racking room floor caused by rolling casks from the filling machine can be minimised by a strip of rubber sheet from each filling head. All casks are numbered and they are stamped at racking with the gyle number, quality and retail price per pint.

The method of filtration described for the recovery of wort from cooler grounds in Section 509 is also applicable to the sediments in fermenting and racking vessels. If this is done rapidly the beer should be little affected in flavour and can be blended off in the same manner as barm ale. It is well, however, to put it through a sterilising filter or resort to pasteurisation. The quantity of beer recoverable in this way is small, but far from negligible, and will usually pay for a suitable plant in a comparatively short time.

(622) Counter-Pressure Racking.

Fully conditioned beers, including lager and bulk-carbonated top fermentation beers, must be racked against a counter-pressure in the racking machine and casks to avoid rapid evolution of CO_2 and fobbing. Since the pressure conditions in the racking cylinder and casks are equalised, so that the beer may flow in gently under gravity, the system is also referred to as isobarometric. In most cases, the beer is drawn from a tank in a cold room and is filtered between it and the racking machine. Methods of filtration are dealt with in the Sections on Bottling, but the use of top pressure alone, as there described, is usually inapplicable on account of the very considerable pressure required to drive large volumes of beer through the filter and against the counter-pressure at the racking machine, particularly when the tanks are on a lower floor, an additional 0·5 lb. per square inch being required for each foot of lift. A racking pump is consequently almost always essential. This pump is frequently referred to as a pressure regulator, since it should accommodate the fluctuations of pressure at the racker caused by removal of filled casks, as well as provide that necessary to drive the beer through the filter and against the counter-pressure. Both piston and centrifugal pumps are used. The latter deliver beer to the racker without the pulsations which may occur with piston or rotary pumps. The piston type is, however, very generally employed on the Continent for naturally conditioned lager beers.

There is usually a top pressure of about 25 lb. on tanks of bulk carbonated ales, but an additional pressure of about 40 lb. may be required at the pump inlet. The fall in pressure at the filter may be about 10 lb. under good conditions, or as much as 35 lb. when the filter has become clogged by considerable use. American beers may be either fully or partially carbonated in the storage tanks (Section 611) but it is usual to complete the saturation or give a "secondary" carbonation and again pass the beer through the chiller at racking. Very little additional CO_2 is required if the beer is already fully carbonated. The Taber pump incorporates a "gas dusting" device for use in these circumstances. A pump of the type illustrated in Fig. 177 is widely used for carbonating and racking. A carbonation pressure of about 60 lb. per square inch is required for complete saturation and production of the high condition required in America, the beer being delivered through a filter to the cylinder of the racking machine in which a counter-pressure of 15–18 lb. is maintained. The high pressure requires about 3‘400 revolutions per minute in the centrifugal pump, but two-stage pumps operating at 1,800

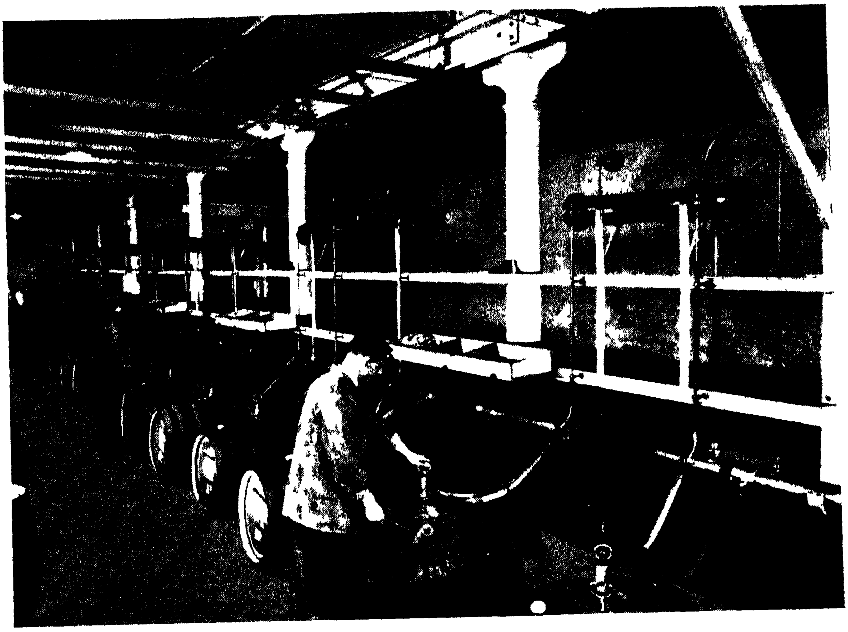

FIG. 182

RACKING FROM SETTLING BACK

(THE BRISTOL BREWERY GEORGES AND CO. LTD. BRISTOL)

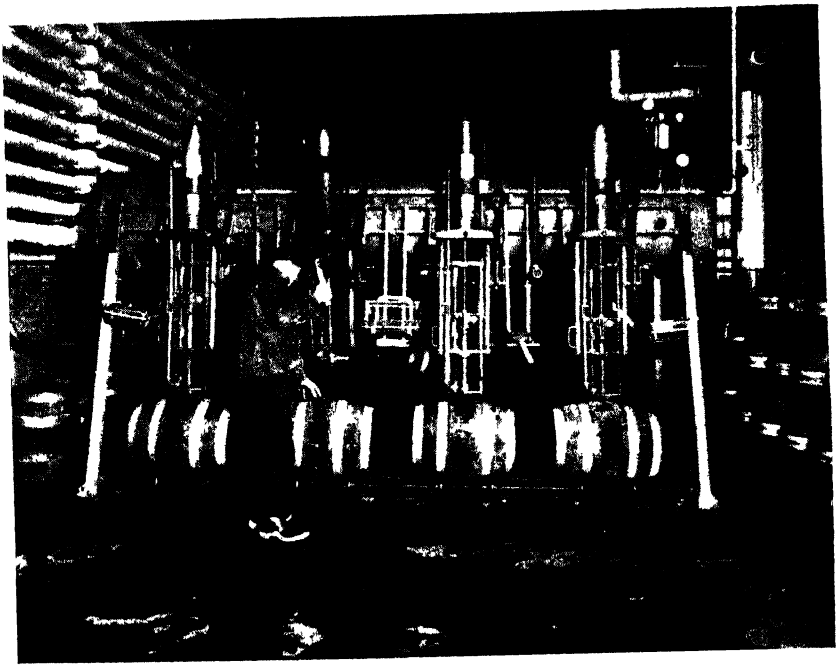

FIG. 183

COUNTER-PRESSURE RACKING MACHINE

SCHREIBER BREWING CO. BUFFALO, N.Y.)

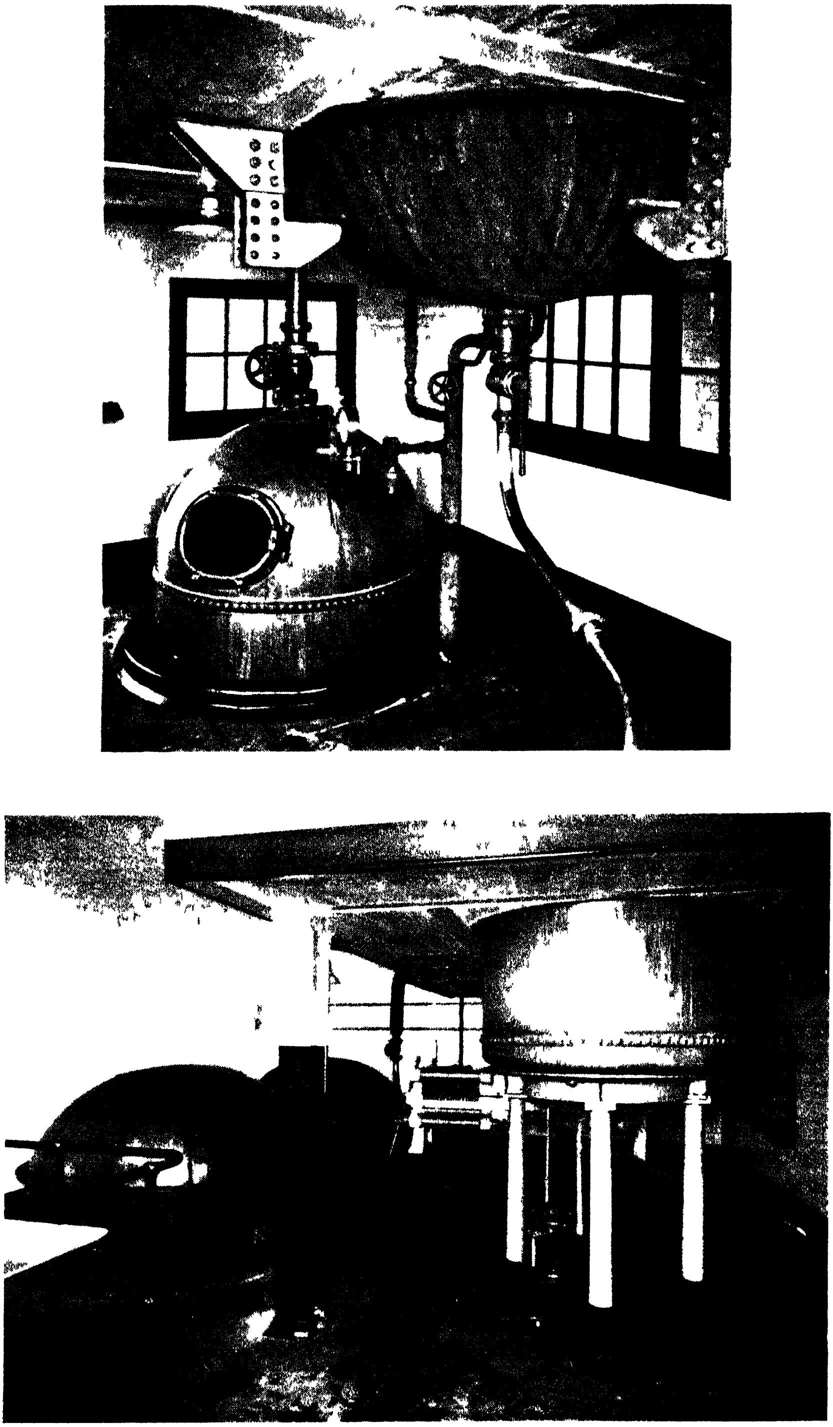

Fig. 184

PRIMINGS MAKING PLANT (WHITBREAD AND CO., LTD., LONDON)

Facing page 865

revolutions per minute are sometimes used. Racking pumps are frequently constructed to blend beers from more than one tank, and are fitted with a sight glass on each suction main. They are mounted on trolleys and must be connected to the tank by hose or mains of ample diameter and placed to avoid sharp bends.

Fig. 183 shows a typical counter-pressure racking machine, the Schlangen racker. The filling mechanism is hinged so that it can be drawn easily over the casks for introduction of the beer pipe which is lowered on telescopic tubes. Automatic valves come into operation when airtight connection is made with the cask, and admit air at 15–18 lb. pressure from the top of the cylinder until isobarometric conditions are established. The beer pipe is lowered through the bunghole until it reaches the bottom of the cask, when its valve is opened automatically and beer flows gently, with very little frothing. A sight glass on the return pressure tube indicates when the cask is full. The filling head is then raised and all valves close. The cask is shived and rolled from the machine. The whole system, from storage tank, through the pump and filter to a by-pass on the racking machine, is filled with liquor to drive out air, and displaced by beer before connection is made to the racking machine.

The principle of racking into casks which have been filled with SO_2, adopted in some Seitz machines, is a refinement of the ancient method of sweetening casks by insertion of a burning " sulphur match " or piece of brown paper coated with sulphur. After the machine has been sterilised with steam, it is cooled and washed with water which has been rendered germ-free by passage through a sheet filter, described later. An SO_2 cylinder is connected to the filling head, and the gas is passed into the cask when the filling tubes are lowered into position. Sterilisation can only occur in a damp cask. Filtered air is then admitted to the cask, drives out the SO_2 and remains as top pressure. The beer valve is operated by means of a lever grip, and the cask is filled as usual with a counter-pressure racker (Section 623), the overflow beer being returned to a special receiver. The time required for sterilisation reduces output by about one-third.

The general use of flexible rubber hose for the connections between the tank, pump, filter and racking machine makes it necessary to consider the possibility of production of a partial vacuum between storage tank and pump, since this causes foaming and suction of air through any loosely fitting joints or connections. This is avoided by putting a top pressure of 10–15 lb. on the storage tank and by use of mains or hose of sufficiently wide bore. A considerable amount of frictional resistance is offered to the passage of liquids through a long pipe. This is increased to such an extent that a partial vacuum is produced if the pipe is of too small diameter for the flow demanded. The frictional resistance to the passage of 100 barrels an hour through 100 feet of hose is equivalent to a head of 22 feet of water, and this is raised to the equivalent of 33 feet in 150 feet of hose, or approximately 14½ lb. pressure. A vacuum on the suction side of the pump, equivalent to about 9 inches, would consequently be formed if it were attempted to pump beer from a tank under these conditions with a top pressure of 10 lb. on the tank. The friction would be reduced to the equivalent of about 11·2 feet head of water if the diameter of the 150 feet of hose were increased to 2 inches. 10 lb. of top pressure would compensate for this and provide an effective pressure of about 5 lb. as the beer approached the pump. In these circumstances there would be no foaming or leak of air in the pipe.

PRIMINGS, COLOURING, DRY HOPS AND FININGS

(623) Primings and Colouring.

Primings are sugar solutions made up to a specific gravity of between 1135 and 1150, which are added to beer in cask or tank to promote condition and give flavour. Invert sugar and sugar candy are widely used, while some of the less fermentable mixtures described in Section 234 are preferred when a slower conditioning is required. Lactose, mixed with three or four times the quantity of cane sugar to provide fermentable matter and sweetness, is used for milk stout. Primings are by no means always used. Some stock beers, particularly pale ales and strong ales, which are stored for considerable periods before sale, depend for their condition on the fermentable sugars remaining in the beer at racking and, to some extent probably, on the breakdown of reducing dextrins to maltose. The choice of a suitable priming sugar depends on the type of beer for which it is intended. Sweet sugars, such as invert, leave a flavour after they are completely fermented, which may not be suitable for all pale and bitter beers. In such cases it may be preferred to use a drier flavoured candy or starch-conversion syrup. The quantity added to the beer also varies very considerably, according to the flavour and condition required. It may be anything from half a pint to a gallon or more per barrel, a quart or two quarts per barrel being very usual additions for the sweeter pale ales, with two quarts to a gallon for mild ales and stouts. No primings are added to the drier stouts, which are frequently "worted" or kräusened.

Great care must be exercised in the preparation of priming solutions, since they readily become a source of infection (Section 644). If the sugar is in casks, and possibly solidified, transfer to the dissolving vessel is assisted by injection of steam, while the cask is placed with its bunghole above the dissolving vessel. 1 kilderkin or 2 cwt. of invert sugar dissolved in 50 gallons should give a solution of about 1145° gravity. The solution is boiled and then passed through an enclosed refrigerator to a previously sterilised copper or stainless steel vessel, in which its quantity and gravity are ascertained for Excise purposes, the sugar dissolving being treated as a brew. A sugar-dissolving plant, designed on the lines described, is shown in Fig. 184. No primings should be left in uncovered vessels, and all solutions should be used as rapidly as possible, no more being made than is required for a day or two.

Caramel is dissolved in a similar manner, but should be made up to a definite standard of colour, as measured by the Tintometer. This can be conveniently done by determining the colour after dissolving, and then adding sufficient water to bring the solution to the standard colour, before the Excise charge is taken. A solution with a colour of 11,520 would be required to raise that of beer by 2 shades, when added at the rate of 1 oz. per barrel. The solution should be tested for permanence of colour and absence of precipitate, when mixed with beer.

(624) Dry Hops.

Dry hops are added to the casks before filling, through a funnel by means of a stick or mechanical screw arrangement. The cones should be unbroken and have a fairly strong but delicate aroma. The flavour and preservative value are not adequately utilised unless the beer is stored for two or three weeks (Section 306). It is consequently almost useless to add them for this purpose to beers that are sold in a few days from racking, in which circumstances they materially add to the quantity of waste beer through absorption, without any counterbalancing advantage (Section 280), apart from the assistance they give to finings and cask cleaning. Examples of their influence on the antiseptic content of beer are given in Table 113, Section 306.

(625) Finings.

Finings are colloidal dispersions of isinglass in acid solutions, used for clarifying beer rapidly. They should be added to unfiltered beers at dispatch from the brewery, and not by the customer. Finings are also added to top fermentation beers intended for filtration, time being allowed for settlement to avoid clogging the filter. Lager beers are rarely fined, the lengthy storage, with or without chips, producing sufficient clarification to save the filter.

The isinglass from which finings are made is obtained from the swimming bladders of several varieties of fish, sturgeon in particular. That from the Russian sturgeon is of highest quality. The type known as "long Saigon leaf" follows very closely. Among other kinds, described by their country of origin and shape, are Penang leaf, Brazilian lump, Penang pipe, East India pipe, China leaf, Maracaybo, Penang and Bombay purse. The swimming bladders of the cod and hake are also used to make cheap isinglass in ribbon form. Skins of the roker or ray are used to some extent on the Continent, either alone or mixed with isinglass. The choice of isinglass is not without influence on the success of fining operations. Some brewers find one type definitely more effective than others, while another brewer would select material from a different source. This appears to depend largely on the brewing liquor. Thus Saigon is more suitable than Penang with very hard or alkaline liquors, while Penang would be preferred when the beers are brewed from softer liquor.

Isinglass consists mainly of proteins, with some 14–16% of water and variable amounts of ash, 0·2–1·5%, and of insoluble matter, 3 to 5% in good qualities. The following figures are averaged from a number of analyses of brewery finings by Harman, Oliver and Woodhouse.[5] Protein 0·82%, ash 0·072% and 280 parts per million of sulphurous acid. The last, it may be noted, will only raise the SO_2 content of the beer by 2 parts per million, when the finings are used at the rate of a quart per barrel. The acid used for solution or "cutting" may be supposed to diffuse into the protein particles, so that protein cations and acid anions accumulate within what may be regarded as a semi-permeable membrane, through which water will pass under the influence of the osmotic pressure set up and swell the protein particles. Hopkins[6] found that the swelling was greatest between p_H 3·3 and 3·5, varying with different kinds of isinglass and acid. It appears that the whole of the protein is converted to an acid salt when the finings are "over-cut," with the result that they become liquid and lose their clarifying power.

(626) Manufacture of Finings.

Cutting or solution is carried out by immersing the isinglass in solutions of tartaric, sulphurous or acetic acid until they are sufficiently softened and swollen to pass through sieves. Formerly the isinglass was cut in acid beer, the mixture of lactic and acetic acid being an excellent medium for the purpose. This method is practically obsolete, being ineffective with the lighter gravity beers of the present day, in which development of the necessary quantity of lactic acid is impossible, and on account of the danger of infection. Acid beers used for the purpose should be sterilised. Solution may be accelerated by cutting the isinglass into small strips and by frequent stirring, the time required with uncut isinglass varying greatly with its nature, and with the acid employed. It may be as long as six weeks with Saigon and only a week with China leaf. Tartaric and sulphurous acids or mixtures of the two are usually considered the most suitable solvents. They cut well and also act as preservatives.

14 lb. of isinglass may be placed in an unheaded hogshead or, preferably, in a glass-lined or earthenware tank, and covered with water. 2 lb. of tartaric acid is dissolved in the water and 2 gallons of sulphurous acid (5%) added. More water is added as the isinglass swells and, when the latter becomes completely softened, with the exception of the skins and some hard ends, it is pressed through sieves with brushes and broken down to 54 gallons. This may be further sieved and diluted to about 6 barrels for use. This should be thick enough to give rapid fining when 2 or 3 pints are added to a barrel of beer. The temperature should not be allowed to exceed about 55° F. during manufacture or storage. A viscosity test, with a funnel, having a small aperture, may be used to check the dilution from the time required to pass a given volume, say 5 oz. The results afford a fair indication of the clarifying power of any one kind of finings, when carried out at a standard temperature, such as 55° or 60° F., but may not accurately compare the properties of finings made from different materials.

A rapid process for making finings in 18 to 20 hours with a patented machine was described by Reavenall.[7] The isinglass is cut in thin strips, 0·5 to 1 inch thick and 4 to 6 inches long. 37 lb. of the strips are placed in a wooden vat of 1,000 gallons capacity. 9½ lb. of tartaric acid, dissolved in a little water, and 19½ pints of sulphurous acid, together with 60–70 gallons of water, are poured over the dry strips and allowed to stand for 5 hours, with occasional stirring. The vat is provided with a stirring device, which is set in operation at the end of this time, when water is permitted to flow in at such a rate that the vat is filled in 12 hours, making a total of 17 hours. A cock at the bottom of the vat is then opened, and the contents allowed to run through a special form of mechanically operated strainer into a collecting tank. The finings produced are ready for use.

(627) Operation of Finings.

The quantity of any particular finings required to clarify beers varies considerably with the beer, its temperature and acidity. Increase from an optimum quantity of a quart per barrel, in a particular instance, to 2 quarts has little if any additional effect and may hinder clarification. Similarly, re-fining of a previously fully fined beer is liable to be ineffective. Fining can be carried out successfully at the low temperature of a cold room, but is accelerated as the temperature rises and is probably most effective around 55° F. or slightly above this. It should always be carried out with a rising temperature. Cloudiness, due to precipitation of protein matter held in solution at higher temperatures, almost inevitably occurs if the temperature of the beer falls after fining. Clarification may also be retarded if the acidity of the beer is too high or too low. " Floaters " may occur in beer which has been racked too fine, unless the quantity of finings is reduced. The coagulum usually settles firmly on the bottom of the casks, but high temperatures cause it to rise, and it may come completely to the surface at 75° F. After settling, the beer would then be liable to become cloudy through precipitation of proteins, as the temperature fell. The coagulum is particularly inclined to rise from the bottom under the influence of CO_2 evolution when the yeast has high clumping properties. The occasional experience of " fining in layers," that is to say, delivery of cloudy beer from the tap after previous drawings had been bright, followed by another flow of bright beer, is to be attributed to such conditions. The coagulum is sometimes allowed to come to the surface with CO_2 and " fine out " through the bunghole. The rather gelatinous deposit tends to become sandy in presence of excess of suspended matter in beer. Primary yeast, even when in fairly active fermentation, does not hinder fining action, unless its quantity is excessive. Some of the smaller types of wild yeast are, however, very resistant to fining, which entirely fails to remove them from a fretting beer. This appears to be associated with lack of flocculating properties in these yeasts and their even distribution throughout the beer. The more highly hopped a beer is, the more difficult does clarification usually become. Thorough rolling or agitation of the cask materially assists fining.

Finings made in the same way from different kinds of isinglass act differently with the same beer at varying p_H values. According to Harman, Oliver and Woodhouse, Penang had the widest p_H range of those tried, so that it should work satisfactorily with any class of beer. Saigon and Indian worked well in more alkaline beers. Brazil and China leaf were of little value, except for beers from acid-treated liquor or carbonate waters which had been thoroughly boiled.

(628) Physics and Chemistry of Fining Action.

Mixing finings with beer corresponds with the dispersion of colloidal proteins in a liquid containing both suspended solid matter and dispersed colloidal particles. Their action was formerly attributed to precipitation of a coagulum which enmeshed and carried down solid particles as it settled in the beer. That this provides a very imperfect explanation is evident from consideration of the principles governing the behaviour of colloids (Vol. I, Sections 83–91), and from experimental evidence. The stability of a colloidal dispersion depends on the charges on the particles. If the charges are neutralised, the particles cease to be mutually repellent, will tend to coagulate and precipitate. This occurs most sharply at their isoelectric point (Section 87), which, in the case of the proteins of isinglass, is at about p_H 5·0. They carry a positive charge at the more acid reaction of beer, but, being lyophilic colloids (Section 89), are not readily discharged and precipitated by negatively charged ions. Harman, Oliver and Woodhouse, indeed, found that finings remained completely dispersed when mixed at the rate of 1 quart per barrel with a buffer solution at p_H 4·0 containing no other colloids.

Another explanation must consequently be found for the coagulation of finings in beer at a p_H between 3·8 and 4·2. Neutralisation may occur to a certain extent through the action of negatively charged ions, particularly the $SO_4^=$ of gypsum, but precipitation is probably much more dependent on neutralisation by the opposite charges of other colloidal particles. It may be supposed that the very minute micro-organisms and other negatively charged particles are adsorbed on the positively charged micells of finings, whereby neutralisation and precipitation occur.

It was stated in Section 90 that a very small quantity of a lyophilic sol added to a lyophobic sol decreased the stability of the latter, but addition of the lyophilic sol in excess renders the lyophobic particles more stable and less liable to coagulation. In other words the lyophobic particles are protected and precipitation is prevented. This state of affairs occurs when excess of finings is added to beer, in which the suspended micro-organisms and other particles behave as lyophobic colloids, the lyophilic colloids of isinglass having a high protecting power. The result is that excess of finings may not only fail to precipitate the particles, but may actually stabilise a haze already present. Harman and his co-workers observed that the quantity of the proteins of finings remaining in a clarified beer may amount to as much as 50% of that added, and that the separation of coarser particles during fining is accompanied by stabilisation of the remaining colloidal solution. Satisfactory fining is thus the result of a nice balance between separation and protection. An increase in the amount of finings results in a larger quantity remaining in solution, so that the rate of fining seriously affects the fining action. Different finings also vary appreciably in their protecting properties.

(629) Auxiliary Finings.

Solutions of Irish Moss are frequently used to increase the precipitating effect of finings. The improvement is due to neutralisation of the charge on the finings particles by the opposite charge on the colloidal particles of the Irish Moss preparation. As a result, some of the finings, otherwise held in solution, are precipitated. The increased amount of coagulum may remove wild yeasts and other haze-forming materials when finings alone were ineffective. The increased precipitation of isinglass protein may also diminish its protective effect. Similar improvement in fining may occur when the Irish Moss was used in the copper, as some of it may pass through to the beer. Washed kieselguhr and chalk have sometimes been used with finings. The former probably acts by neutralising electrical charges and, partly, by weighting the precipitate and speeding its settlement. Its use is not, however, advisable with beer. Chalk neutralises the acidity of beer and probably influences the separation of finings by evolution of CO_2.

CELLAR TREATMENT OF CASK-CONDITIONED BEERS

(630) The Brewery Cellar.

Management of beer in the brewery cellars before it is sent to the customers varies greatly in different breweries, and in the same brewery with different beers, but it is always desirable that the beer should be kept at a few degrees lower temperature than that of the customers' cellars. The finings, added at dispatch, should always be allowed to work in a slightly rising temperature. This places the maximum at 55° F. and the installation of automatically controlled temperature-regulating plant whenever necessary will be found to cut down " summer troubles " or fret and finings difficulties almost to vanishing point with beers that are stored for a few days or a week or two, if proper care is taken after the beer leaves the brewery. A temperature-controlling unit on the loading bank, which is closed with sliding shutters, is an additional refinement which permits assembly of casks during the afternoon for the first delivery in the morning.

The old system of storing beer in the open air for six months or a year, to pass through one or more cask frets and become spontaneously brilliant, is practically abandoned, except for a few special export and strong beers. Even storage for two or three weeks, while the beer passes through its first period of conditioning, with careful management of a porous spile, is becoming a lost art in most breweries. The porous spile is a conically turned peg about 2 inches long, made of such porous wood as the American Black Oak, which has tubular cells running in the direction of the grain along its length, the ends being cut transversely to permit free passage of CO_2. They are knocked into a hole through the shive, and allowed to function so long as it is necessary to reduce pressure in the cask during conditioning, after which they can be replaced by a hard peg. Care is required to avoid flattening. They may also become clogged with yeast and protein matter, so that replacement may be necessary during lengthy storage.

The custom of allowing yeast to purge through the bunghole for a few days after racking is maintained in some breweries. In others, the casks are closed temporarily with a bung and square of boiled hop sacking, but tight shiving at racking is more general. The practice of priming, fining and shiving at racking for immediate dispatch is common with light gravity mild ales, but beers are more frequently stored for a short time. The time may, in many cases, be governed by exigencies of sale, but mild ales are usually kept from three or four days to a week or rather longer while bitter beers are given from five days to two or three weeks. They are finally primed, fined, topped up and re-shived for dispatch. In some breweries, part of the primings is added in the fermenting vessels, after removing the final heads, a helpful practice when cleansing is not very complete. In others, part is added at racking and the remainder, with the finings and, if necessary, colouring, after removal of the required amount of beer from the casks. The practice of fining in the houses is becoming less and less common, as it is generally recognised that these final operations should be kept strictly under the brewer's control.

(631) The Public House Cellar.

Stress has been laid on the desirability of a beer temperature of about 53°–54° F. at racking, to allow for the slight rise in the brewery cellar and during transport to the public house necessary for perfect conditioning and fining. Equal insistence must be placed on the temperature of the public house cellar. 55° to 58° F. is usually regarded as most suitable for British beers. This should be measured by a thermometer hanging from the ceiling, a few feet from a wall. A wall thermometer is often very misleading. Violent condition and cloudy beer may result if the temperature rises above 60°. Such conditions require the installation of a small air-conditioning unit in the majority of cellars for use in the summer and some means of cooling the beer below 53° before racking. Casks of beer sent from the brewery at a temperature of 58°–60° F. must inevitably result in the sale of mawkish beer, or risk of greyness, if the temperature falls a few degrees in the public house cellar. Some means of heating the cellar is generally required in the winter. Unfortunately a controlled heating system, like the automatic refrigerating unit, is expensive and it is necessary to depend in smaller houses on such improvised alternatives as a brick heated by a gas jet, sacking to exclude draughts in the winter, and blocks of ice and a fan in the summer. The air in the cellar must not only be at the correct temperature, but must also be clean and odourless. It enters the cask as beer is withdrawn and, if it carries bacteria, mould spores or contaminating odours, the beer is bound to suffer.

Beer fined at the brewery should reach the customer ready for stillage. The back of the cask is tilted until it is as high as the tap-hole, and a cane peg inserted in the shive to regulate the pressure. The cask is immediately tapped, so that beer can be drawn as soon as its condition is correct and the finings have settled down. Beer pumps or engines are most frequently employed for raising the beer, most modern types being quite hygienic and satisfactory. CO_2 pressure systems must be carefully controlled by attention to the reducing valves on the cylinders, safety valve and pressure gauge. Much consideration has for some time been given to the material of pipes. Lead and tin should be absolutely avoided on account of slight solution in beer which may have remained in contact with them for any length of time, particularly after cleaning with an alkaline detergent. Stainless steel is generally regarded as the best material for pipes. Natural Monel metal is also highly resistant to beer. Doubly annealed glass can be used without danger, but rubber is to be avoided wherever possible.

It is advisable to drain engine cylinders and pipes at closing time, and fill them with cold water until again required, when they are drained and re-connected. Every week the pipes and pump should be thoroughly cleaned. They may, for example, be filled for an hour with a warm solution of ½ lb. washing soda in 2 gallons water. A brush is drawn through and the system filled for ten minutes with a cold solution of ½ lb. salt in 2 gallons water. This is run off and clean cold water pulled through, until it runs clear. The beer taps should be washed and brushed in cold water before use.

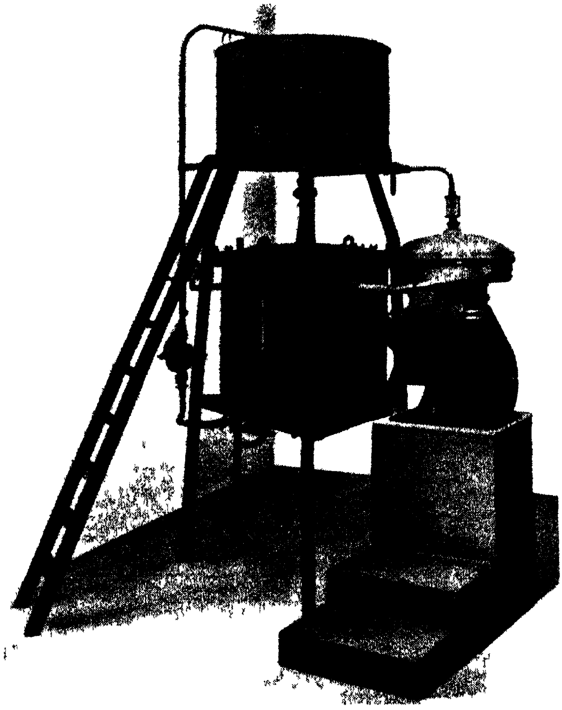

FIG. 185

BEER-SAVING PLANT

(632) Returned Beer.

Defects in public house cellar management increase the returns to quantities considerably in excess of what the brewer would accept as inevitable bottoms. The return of drawings to casks in use should be entirely prevented. It is, indeed, a punishable offence to return beer to any cask, other than that from which it was drawn. The penalty of £50 is the same as that for dilution. The damage to beer and custom liable to follow even the return of filtered beer, offsets the cost of arranging for return of waste beer to the brewery. Returns should be reducible to the small quantity represented by reasonable bottoms, which may average out at about 1 to 1½ gallons per barrel. Such an ideal is, however, generally unattainable in free trade.

The greatest difficulty is provided by frets, whether they be caused by primary or wild yeast. They inevitably cause fining difficulty and the best course is to have casks in this condition returned to the brewery. In some cases a second fining is successful while other beers recover spontaneously and become brilliant in a short time. Some beers, however, become so thin in flavour, develop acidity or other characters which make them quite unsaleable that they have to be regarded, with others damaged by musty casks, etc., as spoilt and claims made for return of duty (Section 530). A considerable quantity of sound beer can generally be saved for blending in the racking square with running ales. For this purpose a special plant for filtration and pasteurisation can be used. A centrifuge and sheet filter are particularly useful and deal satisfactorily and expeditiously with a large proportion of returns. It is also possible to save some acid beers by neutralisation with chalk.

(633) Summary.

Emphasis has been laid on the desirability of a lower beer temperature at racking than is usual in British breweries. 53°–54° F. can usually be reached without detrimental check to fermentation but, in most cases, involves the use of chilled liquor in the attemperators. 54° does not allow much margin for the inevitable rise of temperature at racking, in the cellar and during transport in warm weather. Further cooling between the fermenting vessel and racking back, and air-conditioned cellars may be necessary to ensure that the beer is served at the most acceptable temperature, generally between 55° and 58° F. Low temperature at racking provides for the gradual rise required for effective fining and conditioning and prevents the development of wild yeast frets. It is desirable in winter as well as in summer as a safeguard against the sudden chills which so easily produce cloudy beer, but all the advantages may be rendered of no avail by defective cellar conditions in the public house.

The quantity of healthy primary yeast in beer at racking is another point of importance. On its control depend regularity of condition and stability. The beer must not be too bright, but it should equally not contain so much active yeast as to cause tumultuous cask condition. It is necessary to distinguish between turbidity due to yeast and that caused by an excessive quantity of amorphous nitrogenous matter arising from defective flocculation and sedimentation in the copper or during cooling, which is always harmful.

The ideal of cask sterility can rarely, if ever, be attained. Cask cleaning must, nevertheless, be directed towards it. It is a very good plan frequently to pour half a pint of water into casks and roll them well before returning the water to the glass. Harrow[8] suggested, as a standard of cleanliness, that the water coming out of the cask should be such as one would have no hesitation in drinking. This can be closely approached, if the tests are regularly carried out and necessary improvements in the washing process made according to the results. It cannot be expected that the cleanest water from a cask will prove free from bacteria, yeasts, etc., but their quantity can be so much reduced that a smaller percentage of returns should result. Casks cleaned on rumblers are far better than those cleaned in the ordinary way, and better results are obtained by filling with boiling liquor than by the usual rinse with hot liquor and steam for very short periods. Similar steps should be taken to check the cleanliness of lined casks. Although they are easier to clean than un lined casks, the mere fact of lining is not a sure safeguard.

The number of casks required, three at least for each barrel of beer in a quick trade, is so great and the costs entailed are so large that bulk delivery in tank wagons (Section 612) proves economical in suitable circumstances. Conditioning in tanks is a second alternative which displaces the ordinary cellar conditioning in casks, with much of its risk, and provides a flexible supply of beer for racking in good condition when required for sale.

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MICROBIOLOGY AND BIOCHEMISTRY OF FERMENTATION

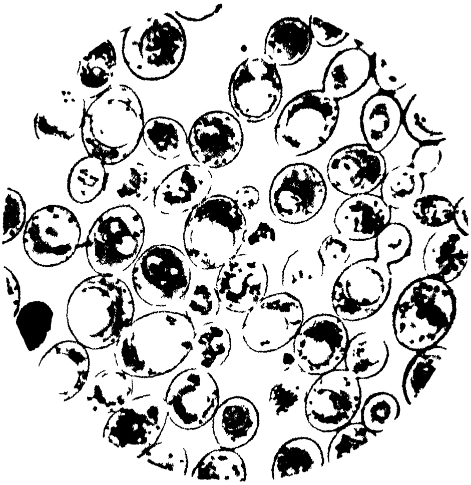


FIG. 186

PILSEN YEAST, LABORATORY CULTURE

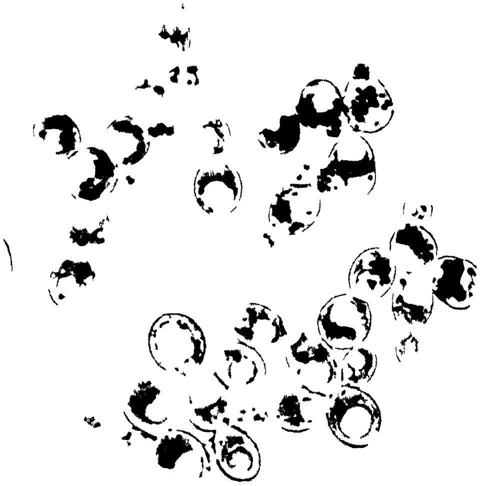


FIG. 187

BURTON YEAST WITH ASCOSPORES

THE RADIATING FORMATION OF CELLS AND SPORES IS TYPICAL OF TOP FERMENTATION YEASTS

BREWERY YEASTS (× 1000)

PHOTOMICROGRAPHS WITH ULTRA-VIOLET LIGHT BY KRUIS AND SATAVA

Facing page 880

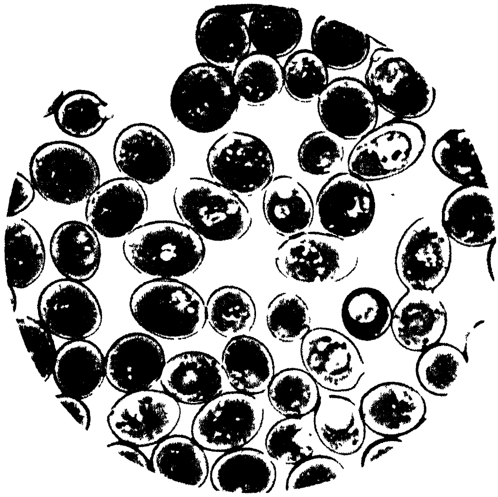


FIG. 188
BREWERY YEAST FROHBERG TYPE

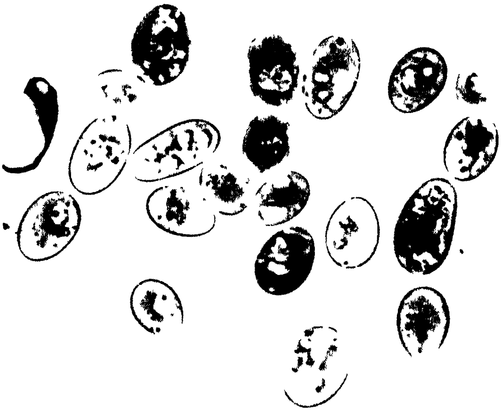


FIG. 189
SAAZ YEAST, FROM AN ORIGINAL CULTURE BY P. LINDNER

BREWERY YEASTS (× 1000)

PHOTOMICROGRAPHS WITH ULTRA-VIOLET LIGHT BY KRUIS AND ŠATAVA

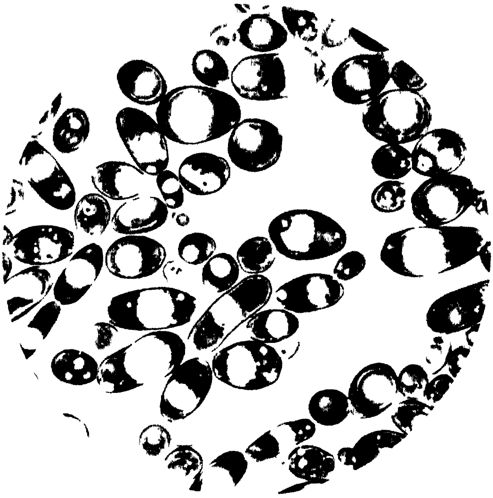


Fig. 190

S. *CEREVISIAE* VAR. *TURBIDANS*

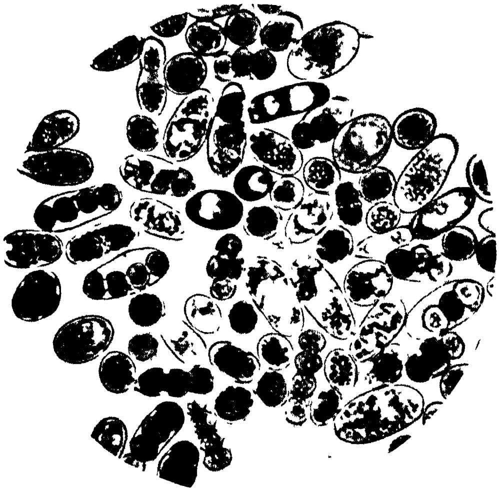


Fig. 191

S. *CEREVISIAE* VAR. *TURBIDANS*
WITH ASCOSPORES

WILD YEASTS CAUSING DEFECTS IN LAGER BEER (× 1000)

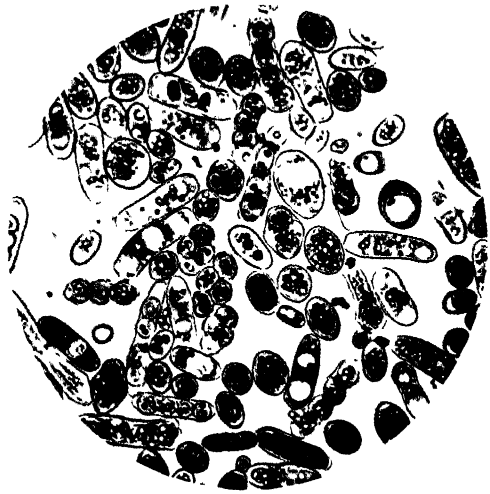


Fig. 192

S. *PASTORIANUS*, WITH ASCOSPORES

FROM AN ORIGINAL CULTURE BY E. C. HANSEN

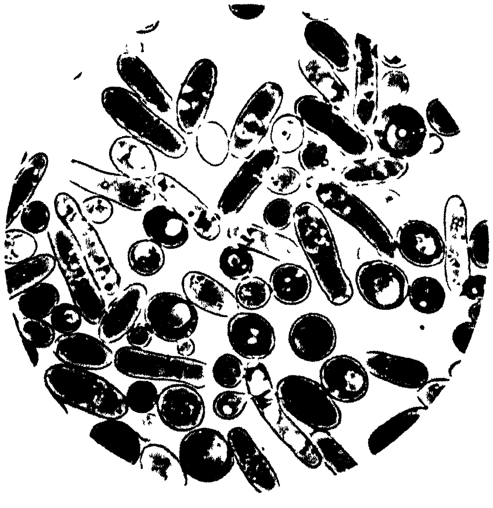


Fig. 193

S. *VALIDUS*

FROM AN ORIGINAL CULTURE BY E. C. HANSEN

WILD YEASTS CAUSING DEFECTS IN LAGER BEER (× 1000)

PHOTOMICROGRAPHS WITH ULTRA-VIOLET LIGHT BY KRUIS AND ŠATAVA

Facing page 881]

CHAPTER XXXVI

YEASTS AND MOULDS

THE YEASTS

(634) Classification of the Fungi.

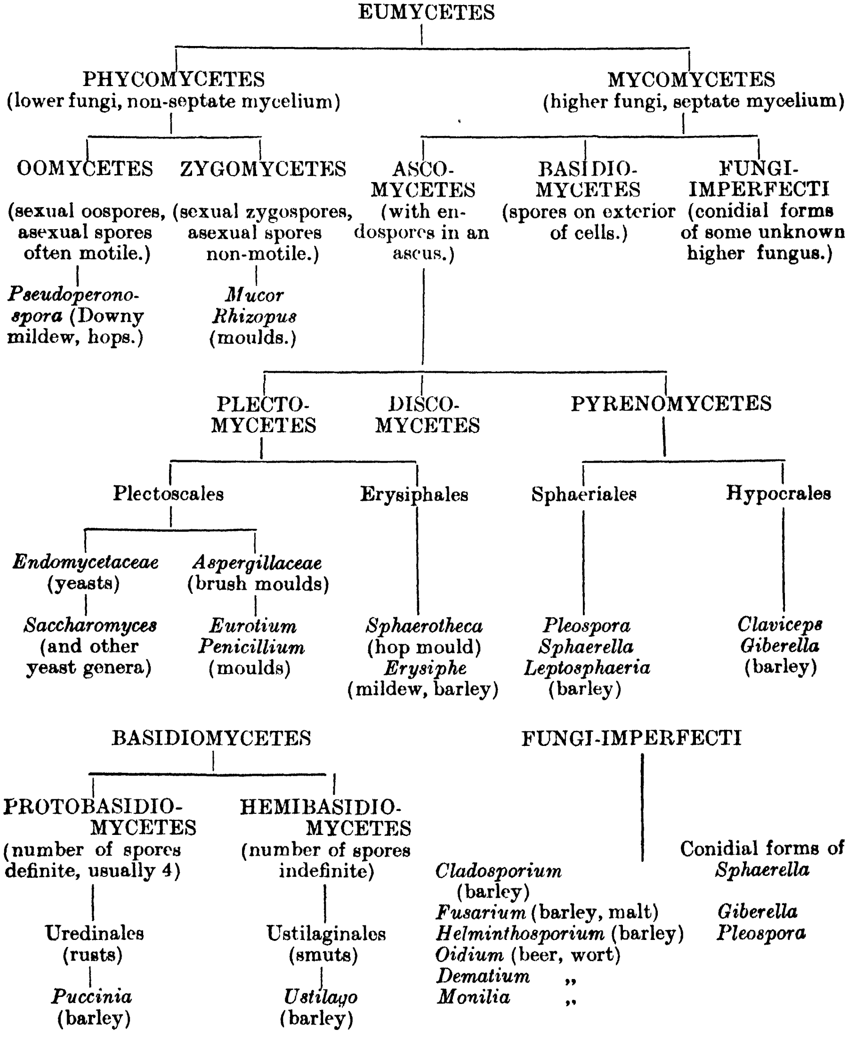
The yeasts, moulds and bacteria are included with a very varied multitude of primitive plants in the lowest primary division of plant life, the *Thallophyta.* Their classification has proved very difficult and the confusion in nomenclature that still exists is a serious handicap in the recognition or description of species that are used in industry or cause damage to raw materials and finished products. The beneficial and harmful may be very closely related in the botanical sense and indistinguishable by their morphological characters or microscopic appearance, so that it is necessary to take their biochemical activities into account as well in schemes of classification. The mass of observation on the races met with in breweries is in a rather chaotic state, and all that is attempted here is to give an abbreviated classification of the groups of "true fungi" or *Eumycetes,* Table 231. This shows the relation between the classes of most of the micro-organisms, apart from bacteria, found in breweries or on barley and hops, with the distinctive characters of the main groups to which these classes belong.

(635) Classification of the Yeasts.

Soon after it became known that fermentation was due to the activity of living, unicellular fungi, which normally multiplied by budding, brewers', bakers', distillers' and wine yeasts were included in a new genus, the *Saccharomyces.* It was subsequently discovered that many yeasts could perpetuate their species under certain conditions by forming spores within the cell, which becomes an "ascus," the spores being known as "ascospores" or "endospores." They were therefore included in the class of *Ascomycetes,* which is characterised by this method of propagation. There are, however, many forms of yeast-like fungi in which spore formation has not been observed or in which spores cannot readily be obtained experimentally. These are still frequently referred to as "doubtful" or asporogenous yeasts to distinguish them from the " true " or sporogenous yeasts. The further classification of both groups is based largely on the pure culture and experimental methods introducted by Hansen.

TABLE 231.—*EUMYCETES* OR TRUE FUNGI

SYNOPSIS OF THE CLASSIFICATION OF THE *Eumycetes*, SHOWING THE RELATIONSHIPS OF THE COMMONER FORMS FOUND IN BREWERIES OR ON BARLEY AND HOPS



The size and shape of the cells under known cultural conditions is taken into account, but morphology is, in most cases, a very uncertain guide, owing to close similarity between different species and the wide variations exhibited under different cultural conditions and even in the same culture of single species. Consideration is therefore given to such biochemical activities as fermentative activity and ability to ferment different sugars, as well as to growth characters, such as sporulation and the shape of the spores, behaviour under different cultural conditions, with film formation and giant colonies. Figs. 205 and 206 are given as examples of the varying appearance of colonies on wort gelatin.

The most recent classification of the sporogenous yeasts is that of Frau N. M. Stelling-Dekker,[1] carried out under the direction of Professor A. J. Kluyver and based on a study of the great collection at Delft. It divides the yeasts into sub-families and tribes, according to the scheme in Table 232, which introduces features not met with in earlier classifications and eliminates some of the formerly accepted genera. Several striking changes in nomenclature will be noted. In particular, the use of *S. carlsbergensis* for bottom fermentation yeasts, *S. cereivsiae* being retained for top yeasts. A number of yeasts previously looked upon as distinct species have been relegated to the rank of varieties of one or the other of these, because they were not considered to possess sufficiently distinct characteristics to warrant their separation as species. Among these are *S. ellipsoideus* and *S. turbidans*, now regarded as varieties of *S. cerevisiae*, while *S. monacensis* is a variety of *S. carlsbergensis*. Species and varieties are further divided into races with still smaller differences.

TABLE 232

RELATION OF GENERA IN CLASSIFICATION OF SPOROGENOUS YEASTS (STELLING-DEKKER)

FAMILY ENDOMYCETACEAE

SUB-FAMILIES

- (A) Eremascoideae
  - GENERA
    - (1) *Eremascus*
- (B) Endomycoideae
  - (1) *Endomyces*
  - (2) *Schizosaccharomyces*
- (C) Saccharomycoideae
  - TRIBES
    - (a) Endomycopseae
      - GENERA
        - (1) *Endomycopsis*
    - (b) Saccharomyceteae
      - (1) *Saccharomyces*
        - (a) *Saccharomyces*
        - (b) *Zygosaccharomyces*
      - (2) *Torulaspora*
      - (3) *Pichia*
      - (4) *Hansenula*
      - (5) *Debaryomyces*
      - (6) *Schwanniomyces*
    - (c) Nadsoniae
      - (1) *Saccharomycodes*
      - (2) *Hanseniaspora*
      - (3) *Nadsonia*
- (D) Nematosporoideae
  - (1) *Monosporella*
  - (2) *Nematospora*
  - (3) *Coccidiascus*

The *Eremascoideae* include forms characterised by a typical mycelium, without any form of sexual multiplication.

The *Endomycoideae* comprise all forms which have a mycelium multiplying by means of oidia. The asci result from iso- or heterogamous conjugation and contain 4–8 round, oval or hat-shaped spores.

The *Saccharomycoideae* include all fungi having a typical mycelium multiplying by conidia-yeasts and capable of producing oidia, and those which are reduced to cell yeasts, budding at all parts of their surface or only at the poles and capable sometimes of producing rudimentary mycelia. The asci, containing 1–4 spores of various shapes, are formed by parthenogenesis, with or without conjugation.

The *Nematosporoideae* comprise forms of which the affinities are still obscure.

(636) The Yeast Cell.

The speck of cytoplasm, enveloped in a tenuous membrane, which constitutes a yeast cell contrives to exist as a single-celled living unit. In many ways it resembles the individual cells of higher plants and animals, but within the compass of 1/3000 of an inch or less, the diameter of an ordinary brewery yeast cell, are collected all the necessary organisations for absorption and utilisation of nutrient materials, synthesis of tissues and growth, including the elaboration of some of the most complicated of substances, such as glycogen, nucleo-proteins and vitamins. Microscopical examination of living yeast reveals little or nothing of the mechanism responsible for this intricate metabolism or for the ordered sequence of enzymic processes that constitute fermentation, respiration and excretion, but more is revealed by appropriate staining methods (Wager[2]) or in photomicrographs taken with ultra-violet light. Counts recorded in Sections 533 and 620 indicate that 1 gram of pressed yeast may comprise 4,000,000,000 or 4,800,000,000 cells. Their specific gravity is about 1·1.

The cell wall consists of two layers, is semi-permeable, preventing the passage of colloidal material but allowing diffusion, entry of the simple molecular nutrient materials and excretion of the products of metabolism—alcohol, nitrogenous substances, CO_2, etc., in solution form. In a young and healthy cell the cytoplasm is dense and almost clear. Within it is a fluid-filled vacuole, containing one or two refractive particles. During the first day or two of fermentation other vacuoles appear, containing glycogen, and these may ultimately almost fill the cell. The glycogen subsequently disappears but reappears when the cells have risen to the surface. A nucleus, closely associated with, but outside the vacuole, and a fine network of stainable material can be made out, granules of fat can also be seen and it is possible to detect the presence of phosphorus and iron.

The ordinary method of reproduction is by budding. A prominence forms on the cell and this rapidly grows. The nucleus divides and part passes to the daughter cell, which separates by constriction from the mother cell, though, sometimes, not until after it has produced a bud in its turn. Although the *Ascomycetes* are a branch of the higher fungi, yeasts do not normally form true mycelia, but the production of threads, consisting of a large number of cells, sometimes occurs in films. In certain circumstances or when budding is prevented by unfavourable conditions, the nucleus may divide in two and these again in two, to form two or four spores. In one genus, the *Schizosaccharomyces*, multiplication is brought about by the formation of a septum in the cell, followed by fission in a manner similar to the multiplication of rod bacteria.

(637) Cultural Characters and Identification of Yeasts.

The cultural characters on which the identification of yeasts depends can only be referred to briefly, but it must be emphasised that the microscopical appearance of the cells of pure cultures is liable to be a very misleading guide. The accompanying photomicrographs show wide variations in form and size in one pure culture. These may become very marked if a yeast is grown in different media or under varying cultural conditions. It is also possible, as Hansen found, to separate strains from one pure culture which differ in behaviour, as well as in appearance, and may retain these varying characters in subsequent cultivations. Henley[3] noted an example of this in strains of *S. intermedius* obtained from the centre and periphery of giant colonies and which, respectively, retained the power of sporulation and film formation or lost both and resembled a torula. Film formation in flasks, half filled with hopped wort and allowed to remain at rest at room temperatures, provides characteristic phenomena with some yeasts. The appearance of the cells in the film may be very different from that of the sedimentary yeast, frequently much longer and suggestive of a mycelium. The limits of temperature within which films are formed, and the optimum temperatures, are stated to be distinctive for different yeasts. Some species are characterised by the shape, structure or colour of the giant colonies developed from single cells on solid nutrient media, such as wort gelatin or agar, and in the appearance of streak cultures. These must, however, also be accepted with caution as diagnostic characters, as they vary widely on different media. Among other characters studied are the behaviour in small flasks of wort or other media, the type and degree of fermentation and aroma produced. The best way to identify yeasts is by comparison under various cultural conditions with pure cultures from a type collection but, even then, confusion may arise from the existence of the same organism under different names given to it by investigators who have unwittingly isolated the same species and submitted specimens to the collection, labelled accordingly.

(638) Spore Formation.

Special interest attaches to spore formation in connection with the identification and classification of yeasts. In nature they are probably formed in circumstances in which vegetative growth is prevented and may serve to tide the yeast over such periods and until normal growth conditions return. Their resistance to desiccation or changes in temperature is not so great as that of bacterial spores, but their occurrence in air-borne dust or in mains and vessels may affect the stability of beer.

The essential conditions for experimental production of spores are a young and vigorous culture of the yeast and a suitable moist surface on which it can be spread and protected from contamination, but with free access of air. The yeast is usually grown in sterilised, bright, hopped wort for 24 hours at 77° F. A little of the culture obtained is then spread on the surface of a small, truncated cone of plaster of Paris, which is placed in a loosely covered glass dish, containing a little sterile water to keep the block and yeast moist. The preparation is placed in an incubator and examined after various periods of time. The time required for spore formation at such temperatures as 77°, 59° and 53° F., the limiting and optimum temperatures, together with the number of spores and their appearance, vary with different yeasts. Instead of gypsum blocks, some workers prefer agar slopes of various composition, such as that of Gorodkowa or Maneval. That suggested by Maneval gives ready spore formation with many yeasts and consists of: beef extract 0·3 gram, sodium chloride 0·5 gram, agar 1·5 gram in 100 ml. distilled water. Carrot and potato slices may also be used.

The spores are usually round, 2 to 4 in number and more highly refractive than the cells in which they are produced, Figs. 191 and 192. Some species are, however, characterised by spores of other shapes. In others they are rather difficult to distinguish or only produced in small numbers. Staining greatly facilitates the detection of spores in shy spore-forming yeasts, hot carbol-fuchsin being frequently employed for this purpose. According to Shimwell,[4] staining with aqueous malachite green is preferable, being much simpler and more reliable with many yeasts. The process is a modification of the Wirtz method used for bacteria. A cover-glass film of the sporulating culture is lightly fixed by passing rapidly through a flame, flooded with 5% aqueous malachite green for 2 to 4 minutes with gentle warming to steaming, washed in water and flooded with 95% alcohol for about 30 seconds. Counterstaining with safranin may be employed if desired, the picture then presented being emerald green spores in a pink ascus.

The development of spores within the ascus of Ascomycetes is held to be of a sexual character, showing rudimentary phenomena that may be regarded as the first steps in the evolution of this biological process. Guilliermond[5] noted two distinct types of conjugation in yeasts. In one, particularly in the *Zygosaccharomyces* and *Schizosaccharomyces*, it occurs before the formation of the ascus. In the other, demonstrated for a number of *Saccharomyces*, it consists in conjugation of ascospores at germination. Conjugation occurs through a canal uniting the two cells into which *Schizosaccharomyces* divide. Fusion of the two cells follows to form an ascus containing 4 to 8 spores.

According to Winge and Laustsen,[6] it is possible to cause spores isolated from two different yeasts to conjugate and thereby produce a hybrid yeast. In the first case of this kind recorded, the smaller spore of *S. validus* united with the larger spore isolated from a bakers' yeast of *S. ellipsoideus* type and a small bud formed at the characteristic angular point of junction. The yeast developed from this bud had certain characters intermediate between those of the parents. This was particularly noticeable in the form of the giant colonies.

This striking demonstration of hybridisation suggests that systematic crossing of varieties or species might be developed to provide a method of yeast breeding, which has hitherto been restricted to selection from types naturally available. Individual spores isolated from one single cell sometimes develop into yeasts of distinctly different properties, so that it would appear impossible to forecast the result of any cross. Winge and Laustsen noted similarities between their hybrid yeasts and the crosses of higher plants, in that spores produced in the yeasts had a very low germinative activity, while the vegetative activity of the hybrid was greater than that of either parent. Šatava[7] has recorded the production of two parallel vegetative forms, one sexual and the other asexual, in *Saccharomyces* and other genera. He noted that the ascospores of certain culture yeasts produced small, round cells, while larger oval cells developed from the vegetative cells, and that this difference was permanent on repeated cultivation. Particularly noticeable was the inability of the former to produce viable ascospores. Hansen's top yeast *S. cerevisiae* was exceptional in that the cells from some of the spores were large. Wild yeasts were characteristically different in that the ascospores normally fused in pairs previous to germination and produced forms identical with the mother cell. The spores, however, occasionally produced "dwarf" cells, but these were not persistent and tended to conjugate.

(639) Selective Fermentation.

No yeast is known to ferment other sugars unless it can ferment glucose, and every yeast which can act on the latter also ferments fructose and mannose. In addition, no yeast is known to ferment both maltose and lactose, but cane sugar can be fermented by yeasts which attack either of these. This varying ability to ferment different sugars is a characteristic property of yeasts, leading to selective fermentation. It is generally believed that the disaccharides must be hydrolysed to hexoses before fermentation which, consequently, depends on the presence in the cell of the requisite enzymes. Thus a yeast cannot ferment cane sugar, maltose or lactose, if it does not contain invertase, maltase or lactase, as the case may be. This manner of explaining the fermentation of disaccharides has, however, been challenged by evidence that some yeasts can ferment maltose directly.

The brewing and commoner wild yeasts all ferment glucose, cane sugar and maltose, but there are several species that cannot attack maltose, though they ferment glucose and cane sugar. Among these are *S. marxianus*, *S. ludwigii* and *S. exiguus*, the two last finding application in experimental fermentations in which it is desired to remove other sugars but leave maltose intact. *S. ludwigii* has also been used in the production of beer with a very low alcohol content. Certain species of *Klöckera* and *Mycoderma* ferment glucose but not cane sugar or maltose. This is not a constant characteristic of these groups and considerable variation in fermenting power exists. Among the Torulae, particularly those found in milk, are some which ferment lactose, galactose and cane sugar but not maltose ; while there are yeasts which have only slight fermenting power and some species of *Pichia* do not ferment any sugar.

An interesting example of selective fermentation, of which use is made in distinguishing top and bottom fermentation yeasts, depends on the absence of melibiase from the former and its presence in bottom yeasts. Owing to this the latter, only, can ferment melibiose and the two types of yeast act differently on the trisaccharide raffinose which, previous to fermentation, is split into fructose and melibiose. Bottom yeasts completely ferment raffinose, but top yeasts can only ferment the fructose formed.

BREWERY YEASTS

(640) Culture Yeasts.

Brewery yeasts have generally all been regarded as varieties or races of *Saccharomyces cerevisiae*, but Stelling-Dekker considered that the top and bottom fermentation yeasts were specifically different and classified them as *S. cerevisiae* and *S. carlsbergensis*, respectively. Mendlik[8] suggested that they have arisen as the result of a process of selection in a yeast that originally contained both types. As a result of the low fermentation temperatures used in lager breweries, certain types persisted, while others, which preferred higher temperatures, were gradually eliminated until practically only the former were left to form the bottom yeasts now in use. Both species include many races, which have been separated as pure strains and, hence, are known as culture yeasts. The characteristics of the races of *S. carlsbergensis* are much the better known, as pure cultures are more generally employed in bottom fermentation breweries. Some of them have been given special names, while others are numbered by the scientific station which has isolated and distributes them. In many cases special strains have been isolated from the original yeast of a particular brewery and, after trial and selection of the most suitable, reserved for use there.

(641) Frohberg and Saaz Yeasts.

In 1889, Lindner isolated two strains of yeast with distinctive properties, one from Frohberg's brewery at Grimma in Saxony, and the other from a brewery at Saaz. These he called Frohberg and Saaz yeasts, which names have come into general use to distinguish strongly attenuating, slowly settling or non-flocculating and weakly attenuating, flocculating yeasts, respectively. Two yeasts with properties characteristic of the two groups were isolated by Hansen from the pitching yeast of the old Carlsberg Brewery and distinguished as Carlsberg No. 1 and No. 2. Later they received the names *S. carlsbergensis* and *S. monacensis* and are described as *S. carlsbergensis* and *S. carlsbergensis* var.

monacensis in Stelling-Dekker's classification. The former has oval cells (7–10) × (3–5) μ, those of the latter are round (5–14) × (5–8) μ. Schönfeld and Rommel characterised other races with similar properties as *Staubhefe* (powder or non-flocculating yeasts), and *Bruchhefe* (break or flocculating yeasts), and these names are frequently used to distinguish the types.

Corresponding types occur in the top fermentation brewery yeasts (Section 556), but it is not yet possible to say how far these are racial characteristics. There are also considerable differences in the morphology of yeasts employed in different breweries, some being typically oval and others round. The permanence or mutability of these types has not been definitely established. A change from one to the other occurs when changes are made between breweries in which either is typical, but this is almost invariably in yeasts that consist of more than one race or type.

WILD YEASTS

(642) Yeasts Producing Defects in Beer.

Hansen used the term "wild yeast" to denote any yeasts which may cause trouble in brewing or produce defects in beer. It implies foreign yeasts which have gained uncontrolled access to the beer and may give rise to defects in brilliance, flavour, etc., during storage. In other cases, particularly in top fermentation breweries, equally uncontrolled infections so constantly accompany the pitching yeasts or are derived from the plant, producing condition during storage or sought-after flavours, that there has been a tendency to regard them as normal partners in the production of certain types of beer and refer to them as "secondary yeasts." It is difficult to justify this view, on account of the liability of these yeasts to get out of hand, and their help in producing cask condition is not required in most modern ales. Since attention was drawn to the grave risks of wild yeast infection, a large number of species have been isolated, described and named, mostly from bottom fermentation beers. The wild yeasts found in top fermentation beers have been so imperfectly studied, that it is difficult to say which, if any, of the species isolated from bottom fermentation beers occur in them. It is remarkable that such an important field of investigation has not yet been systematically explored and that identification with named species must still be based on comparison with isolated descriptions, scattered through the literature of 50 years, or with the more detailed accounts of the wild yeasts found in bottom fermentation breweries.

The forms liable to occur in particular breweries appear to be very constant in type and, in view of this, many brewers feel able to differentiate between those which give rise to frets or turbidities and others which are generally harmless or readily removed with the primary yeasts by finings. In the absence of proper identification, the various yeasts are frequently distinguished as of "*pastorianus*" *or* "*ellipsoideus*" type or by some such description as "small elliptical" or "elongated" yeasts. This is an unsatisfactory state of affairs and may lead to much confusion in view of morphological variations and differences in behaviour under other conditions.

(643) Typical Wild Yeasts.

Five species of wild yeast described by Hansen have become almost classical and the names he allotted to them, *S. ellipsoideus* I and II, *S. pastorianus* I, II and III, have since served to distinguish elliptical and sausage-shaped types. They are now given the following names, the accompanying measurements being those given by Hansen for the limits of size,

S. cerevisiae var. *ellipsoideus* (4·5–9·5) × (2·5–6·5) μ
S. cerevisiae var. *turbidans* (5–12) × (2–9) μ
S. pastorianus (3–17) × (3–8) μ
S. intermedius (4–16) × (2–6·5) μ
S. validus (5–12) × (1·5–5) μ

The accompanying photomicrographs show how each species varies in size and shape, but sausage-like forms predominate in the *pastorianus* types and are much rarer in *S. ellipsoideus* and *S. turbidans*. Stelling-Dekker regarded the last two as lacking in sufficient differences from the top fermentation *S. cerevisiae* to warrant their separation as individual species, for which reason they are classified as varieties of the latter. The first is a typical wine yeast and is not usually regarded as pathogenic to beer. It has oval or sausage-shaped cells, considerably smaller than *S. cerevisiae*. The cells of *S. turbidans*, Fig. 190, are round or elliptical, rarely elongated. It is particularly characterised, according to Hansen, by the readiness with which it forms films, in which the cells are typically longer than those of the sedimentary yeast, and its ready sporulation, with an optimum at 29° C. (84° F.). It is not infrequently found in bottom fermentation beers, giving rise to persistent turbidity.

Two yeasts of similar elliptical type, isolated by Will, are described as dangerous in bottom fermentations and may possibly be found in top fermentation ales. These are *S. willianus* (7·5–14) × (3·5–6·5) μ and *S. bayanos* (7–11) × (5–6) μ. The former was stated to ferment and propagate very strongly, giving a peculiar sweet taste to beer, followed by a rough after bitter with an odour of rotten fruit.

S. thermantitonum Johnson[9] was stated to possess comparatively great resistance to heat and was expected to find technical application in the tropics. It was found to ferment well at 104°–113° F. and to survive 184° F. It has not been sufficiently well characterised to establish its place in the classification, but is placed by Stelling-Dekker with *S. cerevisiae* var. *ellipsoideus.*

Two of the three *pastorianus* yeasts isolated by Hansen are dangerous in breweries. *S. pastorianus,* Fig. 192, was originally isolated from dust in a brewery. Yeasts of this type are often found in bottom fermentation breweries. They impede clarification during storage and give a disagreeable flavour. It is a bottom yeast, fermenting melibiose, predominantly elongated and sausage-shaped, and sporulates readily, with an optimum given as 27·5° C. (81·5° F.). *S. intermedius* is distinguished from the latter by producing a weak top fermentation, but does not appear to be harmful to beer. Most of the cells in a young film are round or oval. *S. validus,* isolated by Hansen from beer sediments, is found in bottom fermentation beers, in which it produces cloudiness. It gives very elongated cells in films and mycelium-like deposits.

(644) Infection of Sugar Solutions.

The danger of wild yeast infection of sugar priming solutions is well known and carefully guarded against. A particular yeast, to which the name *S. festinans* (Fig. 203) was given by Ward and Baker,[10] appears specially liable to develop. It ferments sugar solutions extremely rapidly and produces haze near the surface of primed beer. It forms oval to cylindrical cells (9–19) × (5·5–4·5) μ and does not readily produce spores. According to Ward and Baker, this yeast may spread rapidly through the brewery plant, even passing through beer filters. Thorough cleansing of the brewery plant is necessary if infection is to be eliminated. In so far as this yeast does not ferment melibiose, it can be regarded as top fermenting and has been classified by Stelling-Dekker as *S. cerevisiae* var. *festinans.*

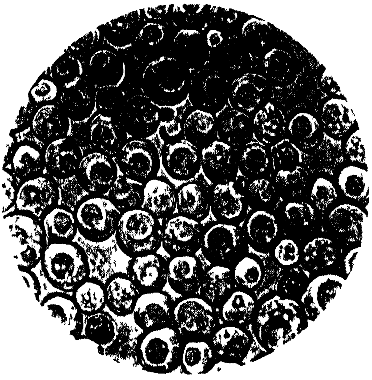


Fig. 194

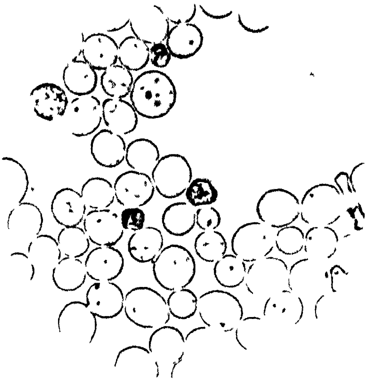


Fig. 195

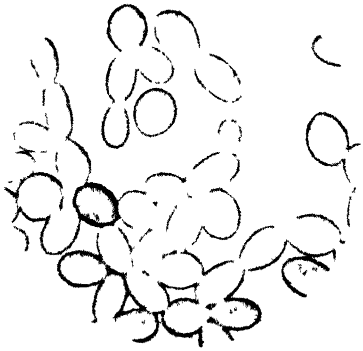


Fig. 196

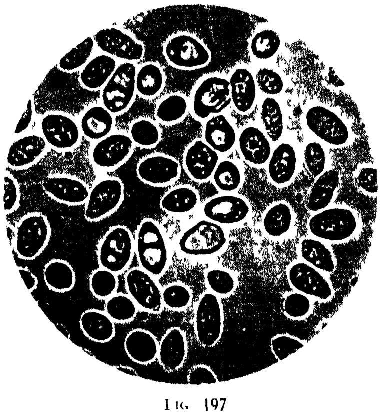


Fig. 197

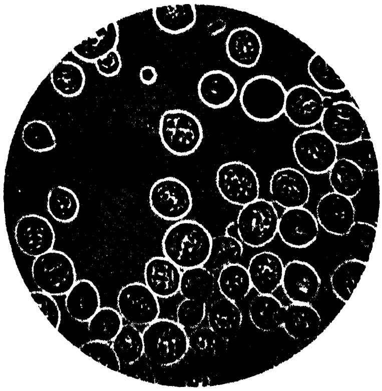


Fig. 198

Brewing Yeasts (× 750)

Fig. 194 YORKSHIRE TYPE

Fig. 195. DUBLIN TYPE (A. T. HENLEY)

Fig. 196. AN EXTREME TYPE USED FOR PITCHING IN ONE OR TWO ENGLISH BREWERIES

Fig. 197. AMERICAN LAGER YEAST (WALLERSTEIN LABORATORIES)

Fig. 198. AMERICAN TOP YEAST „ „

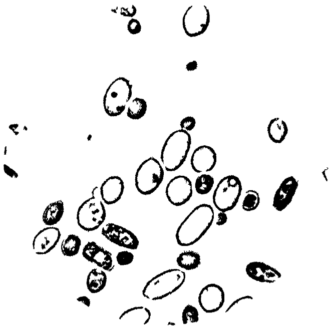


FIG. 199

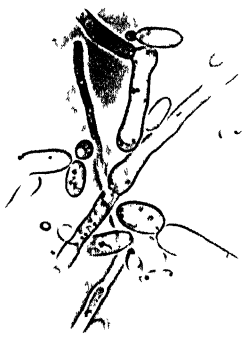


FIG. 200

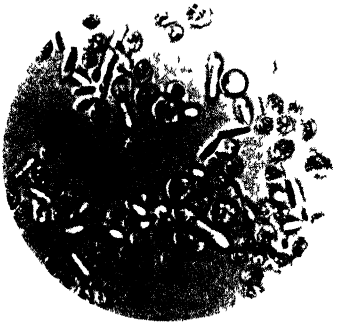


FIG. 201

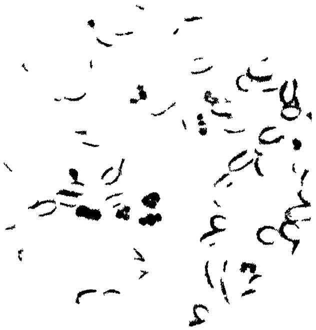


FIG. 202

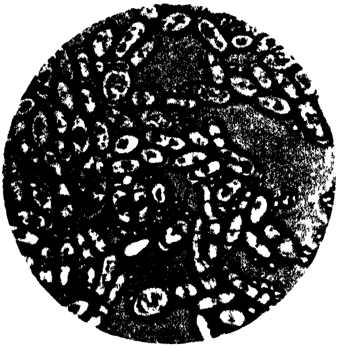


FIG. 203

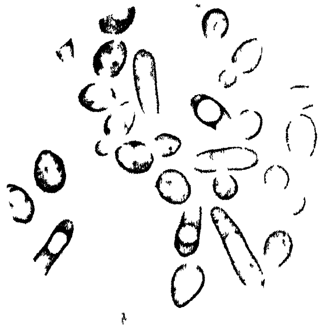


FIG. 204

WILD YEASTS

FIGS. 199 AND 200. *BRETTANOMYCES* (× 750). SUBCULTURES IN WORT FROM SAME CULTURE ISOLATED FROM BOTTLED PALE ALE BY J. L. SHIMWELL.

FIG. 201. *BRETTANOMYCES* AND WILD YEAST IN SEDIMENT OF FORCED BOTTLED BEER (× 500).

FIG. 202. A SMALL SPOROGENOUS YEAST (× 500). PURE CULTURE WITH SPORES STAINED MALACHITE GREEN. ISOLATED BY J. L. SHIMWELL.

FIG. 203. S. *TURBIDANS* (× 680). PERMANENT MOUNT BY T. J. WARD.

FIG. 204. S. *INTERMEDIUS* (× 750). N.C.T.C. COLLECTION. *Phot. A. J. Henley.*

BRETTANOMYCES IN FORCINGS USUALLY HAVE THE APPEARANCE OF CELLS IN FIGS. 201 AND 202 WITH OCCASIONAL ELONGATED FORMS SUCH AS APPEAR IN OLDER BOTTLED BEER SEDIMENTS. THE PRIMARY YEAST AND LARGE WILD YEAST IN FIG. 201 INDICATE THE SIZE.

Facing page 893]

ASPOROGENOUS YEASTS

(645) Torulae.

Pasteur used the term torula for yeast-like fungi which do not form typical mycelia and do not cause alcoholic fermentation. Hansen more strictly defined the group and provisionally limited it to non-sporulating forms. The uncertainty of the division of sporogenous and asporogenous or non-sporulating yeasts is clearly shown by detection, with the aid of special methods of cultivation and staining, of abundant spore formation in some forms which are still generally included in the latter group. Some of these have been placed with the sporogenous yeasts in Stelling-Dekker's classification. Forms included in the torulae are widespread in nature on fruits, in water, etc., and many are of common occurrence in breweries. Most of those isolated by Hansen, Lindner, Will[11] and others appear to be harmless, probably on account of their restricted powers of fermentation, but some give rise to turbidity in weak beers[12] and others have been stated to be essential for production of the special flavours of certain top fermentation stock ales. Many are considerably smaller than *S. cerevisiae*, spherical, and contain a globule of fat, which gives them a distinctive appearance, but others are oval or elongated. Some are characterised by giving yellow or red colonies on solid media. The latest classification, of which that in Table 233 is abridged, is by Jacomina Lodder[13] in a companion work to that of Stelling-Dekker, published under the direction of Prof. A. J. Kluyver.

TABLE 233.—CLASSIFICATION OF ASPOROGENOUS YEASTS (LODDER)

Family 1. *Nectaromycetaceae*, with formation of conidia.

Family 2. *Torulopsidaceae*, without conidia or caratinoid pigments.
  Sub-group A. *Torulopsoideae*, with or without pseudomycelium, no "spore-forming apparatus."
  Sub-group B. *Mycotoruloideae*, with pseudomycelium and "spore-forming apparatus."

Family 3. *Rhodotorulaceae*, without conidia, cells contain caratinoid pigments.

The genera in *Torulopsoideae* are divided as follows :—

(a) *Klöckera*, apiculate cells, bipolar budding.
(b) *Trigonopsis*, triangular cells, budding at the three angles.
(c) *Pityrosporum*, bottle-form or oval cells, budding frequently at the enlarged bases, slow growing in wort with no fermentation.
(d) Cells of other shapes, usually round, oval or cylindrical.

1. *Asporomyces*, on agar, form tubes analogous to the copulation tubes of *Zygosaccharomyces*.
2. *Torulopsis*, no tube formation on agar. Cells round or oval, seldom elongated. May or may not produce fermentation.

3. *Mycoderma*, cells oval or often cylindrical, buds often do not separate from the mother cell. Strong film production on alcoholic liquid media.
4. *Schizoblastoporion*, cells pleomorphic, buds frequently separate from the mother cell.

(646) Brettanomyces.

Claussen[14] (1904) isolated certain non-sporulating yeasts from matured English bottled beers and stouts and gave them the name *Brettanomyces*, because, by inoculation into fully fermented beers and stouts of other kinds, he was able to reproduce the character of the original stock beers. On the other hand, they gave an objectionable flavour when added to incompletely fermented beers. Schiönning[15] later separated a number of types of allied organisms from the ales and stouts of different English and Irish breweries, distinguishing two groups which he called *Torula A* and *Torula B*. *Brettanomyces* species are at present under study at the Centraalbureau voor Schimmelcultures at Delft, with a view to their classification in relation to other similar yeast-like organisms. At the moment sharp differentiation between individual species has not been obtained, but the author understands (through a personal letter to Dr. J. L. Shimwell from Prof. A. J. Kluyver) that a survey of this group may be published in due course.

Organisms of this group may be found in naturally conditioned bottled beers and stouts, when matured for some months or for one month at 68°–77° F., in the form of long slender cells, with small elliptical terminal buds. If some of the sediment is subcultured in a Pasteur flask of wort for 20 days at 90° F. and a plate culture is made on wort gelatin, the majority of the colonies are likely to consist of these organisms and can readily be distinguished from the accompanying yeast colonies by their smaller size. In wort they form small oval cells and not the elongated form. One strain studied by Shimwell (unpublished communication) showed a strong film formation, the film consisting of very elongated cells ; at the same time a very vinous flavour was produced in the beer. This strain showed very varying shape according to the conditions of cultivation, from a "*pastorianus*" appearance in some cases to small oval or almost round, refractive cells in others. It showed a much more rapid growth in beer than in wort. The general morphological characteristics of *Brettanomyces* species, as observed under brewery conditions, suggests that they might be included in the sub-family *Torulopsoideae*. Shimwell found, however, that they are more appropriately classified in the sub-family *Myco-*

toruloideae, since under certain conditions a regular pseudomycelium is produced.

The discovery that the typical and formerly much esteemed flavour of English stock beers was due to *Brettanomyces* gave point to the, then, much discussed possibility of using one selected yeast for the primary fermentation and another for the secondary. In the course of time this has become more of academic interest than practically useful, because very little beer of the particular type concerned is sold and the flavour, which only develops after a period of " sickness " characterised by an objectionable aroma, might, perhaps, hardly be appreciated by a public accustomed to newer beers. In the normal course, the old stock beers went through an early cask fermentation, produced by a mixture of primary and " secondary " yeasts and, after this was completed, other species, probably *Brettanomyces,* which were able to survive concentrations of acidity fatal to the others, appeared to have the power of fermenting dextrins, possibly assisted by diastase contributed by the hops.

The competition between *Saccharomyces* and *Brettanomyces* is complicated by their relative proportions at pitching, but the latter readily overcome the opposition of the yeasts, if present in sufficient quantity at racking and the beers contain fermentable sugars. They can exist in the brewery plant and favourable conditions for their development occur during storage and, even if their activity has ceased after exhaustion of the sugars, they can be revived by priming. They may frequently be found in casks and may sometimes be isolated from pitching yeast, and occasionally develop a haze and stench in running beers. They are not very sensitive to alcohol or acidity and can continue fermentation after *Saccharomyces* are inhibited. They ultimately produce large quantities of alcohol. Schiönning found that his *Torula A* could produce 10% by weight in 6 or 8 months in solutions of sucrose. They produce abundance of CO_2 and give a very firm head to bottled beers. The most prevalent form of *Torula A* was found to be ellipsoid, about $8 \times 4\mu$, but sausage-shaped and even mycelial-like threads occurred. *Torula B* was more slender $(10\text{–}14) \times 2\mu$, but ellipsoid cells also occurred. Old cultures of both consisted of long sausage-shaped, thread-like cells. *Torula B* differed from *Torula A* in that it could ferment lactose. It seems probable that species of the *Brettanomyces* group are a more common cause of trouble in top fermentation beers than has generally been suspected.

(647) Lambic.

Several types of beer produced quite locally are of particular interest from the biological point of view on account of special character derived from the spontaneous development of numerous organisms in symbiosis. Of these, Lambic, a lightly hopped beer produced from barley malt with 40–50% of unmalted wheat, is peculiar to the district around Brussels. It is fermented in casks by organisms derived mainly from the wood, where they have obtained a lodgment during previous use. The microflora has been studied by Kufferath and Van Laer,[16] who have found it possible to reproduce the typical flavour by fermenting with a mixture of a weakly attenuating *Saccharomyces bruxellensis*, two races of *Brettanomyces* and a lactic acid bacterium which they call *Brettanomyces lambicus*, *B. bruxellensis* and *Lactobacillus viscosus bruxellensis*. Normally several *Saccharomyces*, apiculate yeasts, various bacteria and at least two distinct mycoderma are associated with these and the fermentation can be divided in three stages. In the first, the *Saccharomyces* give an attenuation of about 60%. This is followed by development of lactic acid bacteria, producing lactic acid and viscous products. Finally there is an increase of alcohol and acidity in which the *Brettanomyces* are concerned. This spontaneous fermentation normally requires 3 to 4 years to produce the desired beer character, but can be completed in 2–3 months by seeding with the four organisms first mentioned. If *L. viscosus bruxellensis* is added at the commencement of fermentation, the beer does not pass through the viscous stage, but the acid produced favours the development of *Brettanomyces*. The latter ferment slowly with a prolonged lag period and produce a high attenuation, considerable acidity, of which 50–75% is volatile, a compact head and the lambic bouquet. *B. lambicus* gives an unpleasant flavour if used alone, but *B. bruxellensis* produces a pleasant acid flavour. Acid production continues long after alcoholic fermentation has ceased, and the anaerobic conditions in bottle modify the behaviour of *Brettanomyces*. Its production of acid is arrested, but *L. viscosus bruxellensis* continues its activity and facilitates maturation. The whole forms a striking example of symbiosis, without which, as in the case of the British stock ales, the special character of the beer appears to be unobtainable. Metabiosis, in which one organism gains the advantage, may more accurately describe the situation than symbiosis, in which there is mutual benefit in the association.

(648) Apiculate Yeasts.

Among the forms usually associated with the torulae and commonly met with in breweries are the apiculate or lemon-shaped yeasts, formerly grouped as *Saccharomyces apiculatus.* The lemon-shaped cells, 5–10 μ in length, are readily recognised. They are found on ripe fruit and occasionally gain access to beer, to which they may give an objectionable flavour. Many ferment glucose, but not maltose and, consequently produce little alcohol in wort. Hansen associated them with the non-sporulating *Torulaceae,* but spores have been produced in very many races, some of which are grouped by Stelling-Dekker among the sporogenous yeasts as *Hanseniaspora.* Non-sporulating forms are classed as *Klöckera* in the *Torulopsidaceae.*

(649) Mycoderma.

The dull white or grey growth that rapidly forms on beer residues exposed to the air, on casks and sometimes on the surface of bottled beer is usually due to *Mycoderma.* It may contain spore-forming yeasts, among them *Hansenula anomola* and *S. membranaefaciens* but at temperatures higher than 60° F. is liable to be crowded out by bacteria and moulds. Since it thrives at low temperatures and on beer more readily than on wort, it readily develops in cold storage cellars. The *Mycoderma* form no spores on gypsum blocks under the usual experimental conditions, but occasionally produce a very primitive type of sporulating apparatus. The commonest brewery species is *Mycoderma cerevisiae,* Hansen. Its cells are sausage-shaped, (7–10) × (2–3)μ and usually contain three or four vacuoles and two or three highly refractive particles, when grown in wort. The very fine film that forms on culture liquids gradually thickens and detached cells sink to the bottom of the flask. *M. vini,* first isolated from wine, is another common species. The true *Mycoderma* do not ferment maltose or cane sugar and produce little alcohol in wort, unless glucose is present. They may cause a slight turbidity and disagreeable flavour in beer, a small quantity of acetic acid and glycerine from alcohol and a smell of mould in lager beer. According to Jensen,[17] very small quantities of *Mycoderma* in yeast can be detected and estimated by cultivation in a liquid medium containing 1% sodium acetate, 1% ammonium chloride, 0·5% glucose and 0·3% Difco yeast extract at p_H 5·5. 0·1 to 1 ml. of suitable dilutions of the yeast are inoculated in 5 ml. of the medium in a series of glasses, 2·5 cm. wide for fully aerobic conditions, and incubated at 37° C. *Mycoderma* is regarded as absent if no film forms in 5 days. The film must be microscopically examined for verification of the organism. Bacteria are found in the liquid.

(650) Wild Yeast Infection.

Among the most commonly occurring difficulties with which top fermentation brewers have to contend is the failure of cask beer to respond to finings, or delay in becoming brilliant. Occasionally this is due to excessive fermentation by primary yeast (Section 627), but more frequently it can be traced to the development of wild yeasts. Microscopical examination of the deposit centrifuged from persistently cloudy beer or of the yeast collected on a filter paper in very many instances reveals comparatively small oval or elongated cells. Sometimes this is associated with an odour reminiscent of sulphuretted hydrogen, which may only be detectable when the beer is shaken in the glass. Large wild yeasts are also found, but these generally behave more like primary yeasts and are more readily removed by fining. Systematic study of the wild yeasts of British top fermentation beers has been so neglected that it is impossible to say whether few or many species are concerned or to mention the species most commonly occurring. They may be, for example, *S. validus*, *S. turbidans*, *S. festinans*, *Brettanomyces* or other known species, but it is probable that some have not yet been described or named. Fig. 202 is from a photomicrograph of one of the commonly occurring small types. The preparation was stained with malachite green and shows spores, but in many cases yeasts which are indistinguishable in shape and size do not give spores and are, apparently, to be associated with the *Brettanomyces*. In any case definite identification can only be assured by preparation of pure cultures (Section 572), and comparison with named type cultures or published descriptions.

The source of the infecting yeast is, in many cases, difficult to trace. Their original introduction to the brewery may have been aerial, by dust, flies, etc., from external sources of infection, or with yeast supplies. In some cases these may be found by examination of the air, by plate exposures with solid nutrient media. Alternatively and perhaps more frequently the infection may be from some neglected plant where yeasts and other microorganisms may have found a lodgment in conditions favourable to their development (Section 384). Cask infection is also liable to occur and no cask-washing plant can be considered efficient that does not submit the wood to the influence of raw steam or boiling water over a fairly lengthy period.

Treatment of affected beer depends on the virulence of the infection and on the organisms involved. In some cases the fret passes off in the course of a few days with exhaustion of the fermentable sugar and, possibly, of the air introduced at racking.

In others a second fining may successfully remove the turbidity but this can rarely be the case while development is active. Auxiliary finings are in some cases very helpful. The ultimate condition of the beer very frequently depends on its original gravity. Stronger beers may recover and, in some cases, develop attractive flavour. Weaker beers are generally useless after a serious development of wild yeast and may become acid on account of lack of resistance to bacteria. Adjustment of the p_H of the beers at racking and increase of hop rate are unlikely to prove effectual in preventing wild yeast development. Unremitting attention to the plant and sterilisation of all mains and other parts that can be treated with boiling water or steam is essential.

(651) The Forcing Tray.

More than sixty years ago Brown[18] devised the "forcing system" with the object of putting "representative samples of beer under such conditions of temperature as would hasten the development of any of the adverse bacterial changes to which the beer was liable when stored under ordinary conditions." This system of forecasting the behaviour of beer has proved of the greatest value, stimulating the growth of wild yeasts as well as of bacteria. Samples taken at racking may be placed in sterilised flasks of about 100 ml. capacity, with side tubes dipping into mercury or some other form of mercury valve to permit of the escape of CO_2 and prevent ingress of air, or in almost completely filled stoppered bottles. These are placed in a thermostat at 80°–85° F. or on a copper "tray" or flat vessel containing water, thermostatically controlled at the desired temperature. The sediments of the samples are usually examined after 7, 10 or 14 days. The very marked lag phase, characteristic of the development of many organisms (Section 560) must be allowed for. Seven days is frequently insufficient to give a true idea of those present, but 10 or 14 days is generally adequate. A temperature between 80° and 85° is usually employed. The optimum for different yeasts and bacteria varies, however, and it may occasionally be desirable to adjust the temperature to that appropriate to some particular organism for which search is being made. In other cases it may be helpful to incubate 10 ml. of the beer in 90 ml. of some special medium. The indications obtained may be of comparatively little value with any particular sample, but of the utmost service in detecting a dormant infection that might become virulent in favourable circumstances if appropriate steps are not taken to combat it.

MOULDS

(652) Growth of Moulds.

The absence of chlorophyll is a characteristic feature of fungi, that renders them unable to produce their own carbohydrates from CO_2 and water through the agency of sunlight. They must consequently derive their requirements by growing parasitically on a living host or from ready prepared carbohydrate material, for example from the dead cells of other organisms, in which case they are known as saprophytes. The fungi responsible for Smut and Rust of barley are " obligate parasites," since it is only under abnormal conditions that they can be induced to grow apart from the plant. They do not develop on the malting floor. The " black blight " of barley, *Cladosporium herbarum*, is, on the other hand, a " facultative parasite," since it can also live on meat, decaying vegetable matter or on growing malt. A third group of " obligate saprophytes " cannot grow on living plants or produce a disease in barley. Among these are most of the commonest moulds.

The moulds develop preferably in dark places, where the necessary warmth and moisture conditions exist. In the first stage of vegetative growth, a mycelium of long thread-like tubes or hyphae spreads over the surface from which nutriment is obtained. In the next, or reproductive stage, various fruiting organs develop from the mycelium and lead to the production of spores. These are carried off by currents of air, in which they float with dust. In this way they readily gain access to a brewery. The majority do not grow in beer, on account of lack of oxygen, but produce such large masses of mycelium on damp walls, vessels, casks or exposed yeast that they make themselves very apparent. They may not be so harmful as accompanying yeasts and bacteria, but their occurrence provides an outward sign of the probable presence of more serious infections. Their growth is a constant danger in improperly cleaned casks or wooden vessels, particularly in uncleaned, empty casks. The hyphae penetrate into crevices and minute tubes in the structure of the wood, from which they are extremely difficult to dislodge, and communicate a fusty flavour to beer, though damage of this kind may, in some cases, be due to accompanying bacteria.

The moulds contain proteolytic, amylolytic and other enzymes which are, in some cases, so active that commercial use is made of them. Various species belonging to the genera *Mucor*, ***Rhizopus*** and *Eurotium* are capable of saccharifying starch and of fermenting the sugar formed. In the Amylo process for the production of

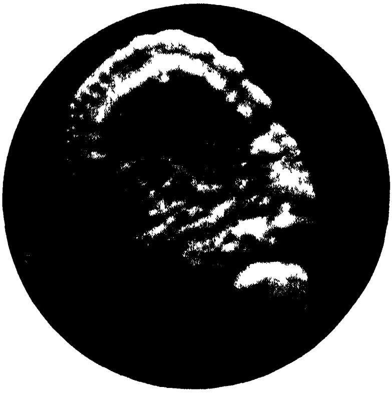


Fig. 205

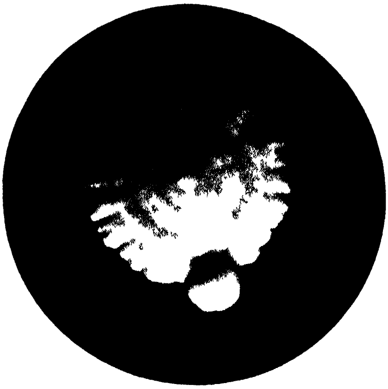


Fig. 206

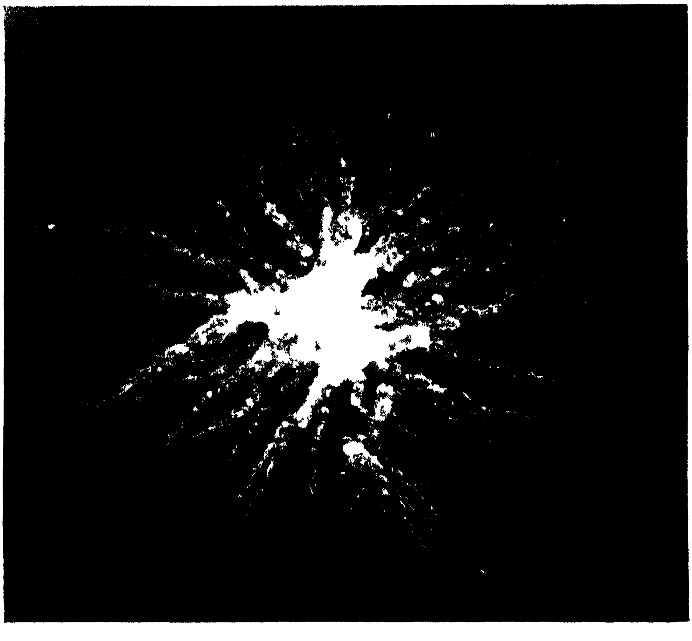


Fig. 207

GIANT COLONIES OF YEASTS ON WORT GELATIN

FIG. 205. *S. CEREVISIAE* (× 4). (F. J. WARD.)

FIG. 206. *S. PASTORIANUS* (× 4). (F. J. WARD.)

YOUNG COLONY ON WORT GELATIN

FIG. 207. *DEMATIUM PULLULANS*. (J. L. SHIMWELL.)

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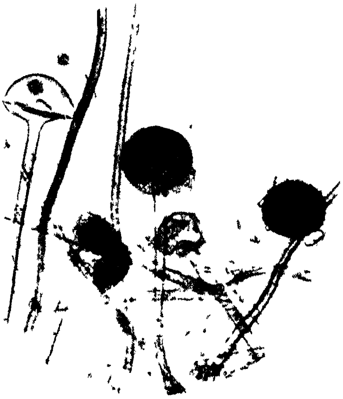

Fig. 208.

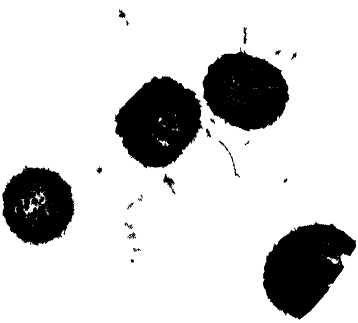

Fig. 209.

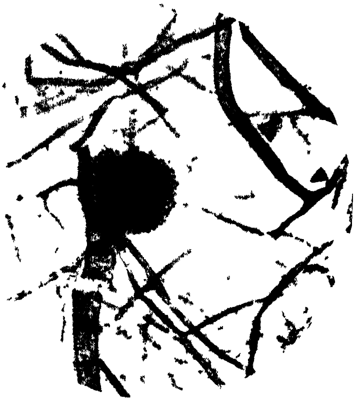

Fig. 210.

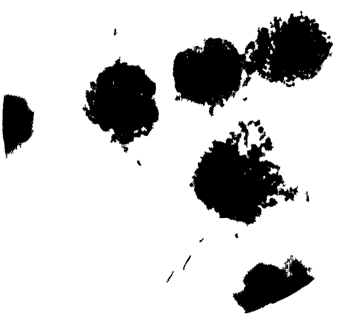

Fig. 211.

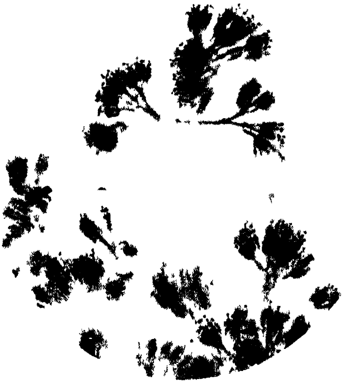

Fig. 212.

MOULDS. *Phot. J. G. Bradbury.*

Fig. 208. *Mucor mucedo* (× 90), sporangia and columellae.
Fig. 209. *Mucor mucedo* (× 90), zygospores.
Fig. 210. *Rhizopus nigricans* (× 90), black sporangium.
Fig. 211. *Eurotium* (*Aspergillus*) *glaucus*, conidiophores with sterigmata and conidia (× 240).
Fig. 212. *Penicillium glaucum*, branched conidiophores, sterigmata and conidia (× 240).

industrial alcohol from grain, the latter, usually maize, is gelatinised by boiling under pressure with water containing a small quantity of acid. The mash is aerated and inoculated when the temperature is reduced to 104° F. with an active culture of the spores of such a mould as *Mucor Rouxii,* also called *Amylomyces Rouxii,* which grows very rapidly when a current of air is passed through the mash at this temperature and at a suitable p_H value. When saccharification is completed, the mixture is cooled to 90° F. and inoculated with yeast, which completes the fermentation more rapidly than the mould alone. In the Boulard process the *Mucor* and yeast are added at the same time and fermentation is completed in 48 hours.

(653) Typical Moulds.

Some mould growths can be identified by the appearance of the mycelium. In some, the hyphae are continuous, *Mucor* and *Rhizopus,* but they are more frequently divided by septa. The methods of reproduction are very varied and some species have more than one method of forming spores. The fructification provides a useful means of identification and special terms are used to describe the different forms or manner in which the spores are produced. Some of these are illustrated in the photomicrographs of a few of the commoner moulds found in breweries or referred to in the scheme of classification, Table 231.

The Downy mildew of the hop (Vol. I, Section 259), and two genera of common moulds, *Mucor* and *Rhizopus,* are listed under the *Phycomycetes* or lower fungi. Several species of *Mucor* and *Rhizopus* are used in the technical production of alcohol and some of the former genus are interesting on account of the yeast-like cells which can be obtained from them by cultivation in wort, through which a stream of CO_2 is passed. *Mucor mucedo,* Fig. 208, is one of the most striking members of the genus. Its mycelium and fructification are frequently seen on animal excreta and it is sometimes found on pressed yeast and green malt. Two forms of spore formation may be observed in this genus. In the most typical, branches, known as *sporangiophores,* grow upward from the mycelium and swell at their apices into globular *sporangia.* The sporangiophores become separated from the supporting branches by septa and develop into short columns within the sporangia which become filled with spores. When these are liberated the *columella* remain, like minute toadstools, with collars round their bases. Occasionally the tips of two branches swell and conjugate, finally fusing into thick walled, warty *zygospores,* which germinate after a resting period, sending up a germ tube and sporangium. Another common species is *Mucor racemosus,* with branched sporangiophores, which develops on bread and vegetable matter. The use of *Mucor Rouxii* in the production of alcohol was mentioned in the last Section.

Rhizopus is a closely allied genus including several common moulds. *R. nigricans* (*M. stolonifer*) produces very striking, spherical, black sporangia, from a yellowish black mycelium. *R. oryzae* is used for saccharifying rice in the preparation of Saké, while *R. delemar* may be employed to obtain alcohol by the Amylo process. It can ferment mono- and di-saccharides and also raffinose or produce 2·7% of alcohol from sweet wort. The fermentative action of these species of *Mucor* and *Rhizopus* is very similar to that of yeast, although zymase has not been isolated from them. They may develop in wort to which spores have gained access, but cannot withstand the competition of yeast.

The group of *Aspergillaceae* comprises a number of closely allied species, sometimes referred to as "brush moulds" on account of the brush-like form of the fructification. The white, bluish-green or brownish moulds that sometimes develop on growing malt are usually among the forms included in the genera *Eurotium* and *Penicillium.* When they grow on malt, they make use of some of the carbohydrate material of the corns and thus reduce the extract obtainable. They also damage the appearance of the malt, but do not appear to affect the flavour of the beer made from it. *Aspergillus* was the name given to moulds which were afterwards found to be conidial forms of fungi included in the genus *Eurotium.* Both generic names are used, but the latter is to be preferred. They are characterised by a septate mycelium and fructification consisting of conidiophores with expanded apex, bearing conidia. *Perithecia* or minute yellowish brown spheres, consisting of a mass of septate threads, in which asci each containing 8 spores develop, are much more rarely seen.

Eurotium (*Aspergillus*) *glaucus* is the commonest of the group and may be found growing on malt or any damp place where nutrient material exists. It may also give a brown colour to hops. The flask-shaped heads of the conidiophores carry a large number of oblong *sterigmata,* each with a string of bead-like conidia, the last formed being at the base of the string. These constitute the greenish dust on a mass of mould mycelium. Several species of this genus are employed technically on account of the active enzymes they secrete. *E. glaucus* serves as the source of Taka-diastase and is used for saccharifying rice in the production of Saké. *E. niger,* so-called from its black conidiophores, converts tannin to gallic acid. It also produces oxalic and citric acids as intermediate products in the decomposition of sugars in the presence of suitable mineral nutrients. *Penicillium glaucum* is amongst the commonest of moulds. Its spores are almost always to be found floating in the air and produce green colonies on wort gelatin exposed in Petri dishes and felt-like, white or bluish-grey masses on growing malt or any place where suitable nutrient exists. The usual fructification (Fig. 212) consists of conidiophores, rising vertically from the mycelium. These are branched at their terminal cell, somewhat in the manner of a bunch of fingers, each of which throws up several pointed cells or sterigmata, which in their turn break up into spherical conidia, which gave a greenish or bluish-grey colour to the mass of mould. If these are blown or fall on to a moist place, they germinate with great rapidity.

(654) Mildews, Rusts and Smuts.

Many forms of fungi which rapidly form delicate white mycelial films, somewhat in the nature of dew, on both living and dead matter when favoured by dampness and warmth are popularly known as mildews. This term has no systematic significance and is applied to fungi of various groups. Two mildews, referred to among the diseases of barley and hops (Sections 52 and 260), are *Erysiphi* and the hop mould, *Sphaerotheca humuli.* Like *Eurotium* and *Penicillium,* they produce perithecia containing asci. Downy mildew is described in Section 259. The Rusts and Smuts are shown in Table 231 as belonging to another group, the *Basidiomycetes,* characterised by a special type of conidiophore, known as the *basidium* or basal structure from which basidiospores grow out.

(655) Fungi Imperfecti.

A large number of fungi, for which a place has not yet been found in the system of classification, are provisionally placed in a group called *Fungi imperfecti.* Many of these are believed to be alternate forms of unknown species and the relationship of some formerly included in the group to higher fungi has been traced. The name *Cladosporium herbarum* has been applied to several similar fungi in the group, but it has been found that one of them was an alternate or conidial form of *Sphaerella tulasnei.* The white, olive green or, later, brown mycelium throws up thread-like conidiophores on which conidia develop, very much like buds. These are olive coloured, septate cells (18–20) × (8–10)μ, which may give rise to secondary spores, closely resembling yeast cells. The " black blight " of barley is ascribed to *Cladosporium*, the development of which on malting floors may be due to spores existing in the barley. The red mould commonly seen on growing malt includes a number of moulds known as *Fusarium* and a *Calonectria.* They are believed to be conidial forms of fungi that give rise to " rot " or " blight " on growing barley and develop from conidia which have survived on the barley. One of the *Fusarium* has been found to be a conidial stage of *Giberella saubinetti,* to which the " head blight " of barley is commonly due and which is dangerous to pigs fed on blighted grain. Development of red mould on growing malt leads to a loss of germinating power and uneven growth. The conidia are crescent-shaped and septate, varying in size according to the number of septa, from (35–45) × (5–5·5) μ, with three, to (60–75) × (4–5) μ when six septa are present.

Monilia candida frequently forms a white mycelium on cow dung and fruits. Should it gain access to wort, it forms cells which are very similar in appearance to yeast cells, can cause alcoholic fermentation and, at the same time, produce a film on the surface. The mould forms threads of elongated cells, with several oval, yeast-like conidia at many of the joints. The conidia of *Oidium lactis* or *Oospora lactis* are cartouche-shaped, parallel-sided cells, with rounded ends. They are sometimes found in wort or beer and may occur on yeast, on which they throw out mycelial threads. As the name implies, the species is more important in milk and is believed to assist in the ripening of cheese. *Dematium pullulans* forms a branched mycelium on fruits and the yeast-like, oval conidia are sometimes found in wort and beer. Growths on gelatin plates cause liquefaction of the substrate. *Dematium albicans* Laurent and *Oidium albicans* Robin are synonyms of *Endomyces albicans,* the name accepted by Stelling-Dekker, by whom it is classed as a sporogenous yeast. According to Jensen[17] *Oidium lactis* can be detected in yeast and its quantity estimated by plating suitable dilutions in a medium containing 1% sodium acetate, 1% ammonium chloride and 1% agar, with 0·1% KH_2PO_4, 0·01% $CaCl_2$, 0·03% $MgSO_4 \cdot 7H_2O$, 0·1% NaCl and 0·01% $FeCl_3$. Colonies of *Oidium* develop but yeast does not grow in this medium. Certain bacteria also yield small colonies, but these are readily distinguishable. The plates are incubated for 4 days at 30° C.

(656) Dry Rot of Wood.

The decay of wood is usually a combined biological, chemical and physical process induced by living organisms of various kinds. In it the compact cellular structure of the timber is broken down into a mass of structureless substances without cohesion or rigidity. Moulds penetrate into wood mainly through open cavities of the wood vessels and sometimes through the cell walls. The principal fungi concerned in the destruction of wood are *Merulius lacrymans* (the dry rot fungus), *Lenzites saepiaria* and *Conidiophora puteana.* The dry rot fungus produces millions of spores which on germination give tubes developing into a mass of very fine threads or hyphae. As these penetrate the open cavities of the wood vessels they secrete enzymes which break down the strengthening lignin, producing soluble substances used for nutrition of the fungus, and leave a friable mass of cellulose. It is believed that a large number of enzymes are secreted from various fungi making them capable of decomposing any kind of vegetable tissue. Zeller states that *L. saepiaria* secretes at least twelve. Destruction of this kind may become unexpectedly dangerous since it may not be noticed until the fruiting stage of the fungus shows at the surface. Special disinfectants are available for destroying the fungus, such as mixtures of salts of nitro-cresylates and fluorides which are very active in dilute solutions. Damage from moulds may be guarded against by dipping the timber in 6% solutions of sodium carbonate or by steaming at a temperature of 170°–180° F. while much can be done by stacking the timber so that there is free access of air.

(657) Summary.

There has been no really systematic investigation of the wild yeasts found in British beers. The effects of their development are only too well known, but it is at present impossible to distinguish the causative organisms by name. Classification based on botanical features is essential as a key to the species, but it brings together races that are vastly different in their behaviour in beer, such as *S. cerevisiae* and *S. turbidans*, the latter becoming *S. cerevisiae* var. *turbidans.* Further, it is exceedingly difficult or sometimes impossible to identify strains from the same original culture which have been grown under different conditions or to identify unknown forms with published descriptions, the variations in form and behaviour are so great. Yeasts that readily form spores may, for example, become quite incapable of sporulating. The writer has been struck with the frequency with which small oval or elongated types develop in casks or forcings and are associated with an odour that recalls the characteristic effect of *Brettanomyces.* It may be necessary to shake samples taken from casks to detect this, but it suggests that species allied with the non-sporogenous yeasts are not infrequently the cause of defects in beer. It is, however, quite impossible to distinguish them by microscopical examination from other species which readily give spores and are, perhaps, more frequently the cause of the serious wild yeast frets and fining difficulties that occur in cask. It should be emphasised that there is no certainty that the best known, named species, mainly derived from lager beer by Continental investigators, are those which give the greatest trouble with top fermentation beers. It may seem of little importance to be able to label a wild yeast by name, but identification must be a first step towards effective counter-measures and the lack of means to do this calls for systematic study of the wild yeasts affecting beer. The literature is full of isolated descriptions of yeasts and moulds, but the mass of unconnected descriptions is so vast that it leads rather to confusion than helpfulness and many of them may apply to the same species isolated and studied under different conditions.

The selection of pitching yeast by microscopic examination of living preparations in water is such an important part of brewery routine that care should be taken in the selection of a suitable microscope and the proper way to use it studied. An enlarged image of a transparent object, with no very distinctly marked details of structure, is formed largely by diffraction of light at its boundaries and at such details as exist. It is difficult to decide whether this image is a true picture of the object or how much of it is the result of diffraction effects, such as those produced by a speck of matter in a brilliant illumination, which would give an entirely distorted impression of the object itself. The implications of this in yeast examination can be judged by manipulating the substage iris diaphragm of the microscope. If the latter is widely opened, the cells become almost invisible and structureless. They become more distinct as the iris is closed until the cells appear to be full of granules, enclosed in what might be thought to be thick cell walls surrounded by a halo of light, as shown in Fig. 198. The image gradually changes, as the illumination is reduced, from the delicately marked vacuole in clear protoplasm, apparently surrounded by a very tenuous cell wall, characteristic of healthy yeast, to a granulated, thick-walled representation of an old or dead cell. Such an experiment demonstrates the importance of constant use of the same exactly judged lighting. Greater or smaller evidences of distorting diffraction effects can be seen in all photomicrographs of living yeast by transmitted white light, since it is practically impossible to produce a distinct picture without them.

There can be no question of adopting what microscopists call "critical illumination" in the examination of living yeast cells, though it should be used in the study of stained preparations, particularly bacteria. What this means is diagrammatically illustrated by Fig. 213. A parallel beam of light is reflected by the flat side of the mirror, entirely to cover the aperture of the substage condenser, by which it is focussed on a point on the object under examination. The objective is also focussed on this point and, by means of the eyepiece placed in a definite position, produces an inverted image at the point of observation.

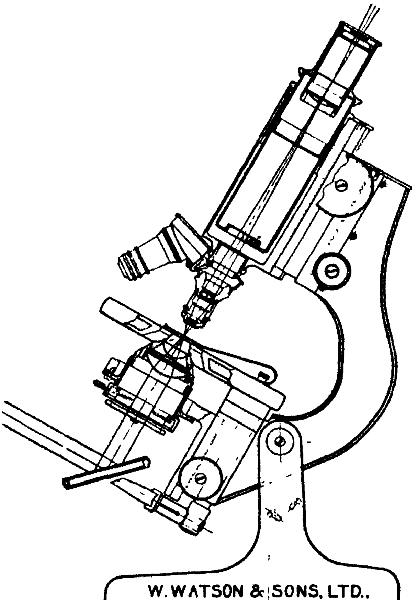

Fig. 213

DIAGRAM OF MICROSCOPE AND ILLUMINATION

The most clearly focussed image can only be produced by using a light filter, passing the monochromatic rays for which the condenser, objective and eyepiece are corrected. Objectives inadequately corrected for chromatic aberration give a coloured image of a colourless object. Yeast cells may appear faintly blue, for example. Any deviation from these conditions of illumination, or use of imperfectly corrected lenses, is bound to produce distortion in the image of the point of detail examined, or cause the details to merge one into the other and become indistinguishable.

The problem with living yeast cells is caused by their extreme transparence and the consequent readiness with which their delicate details are "drowned in light" or made invisible by brilliant illumination. The most correct image is produced by making the least possible deviation from critical illumination by introducing only such amount of diffraction as will make the details visible, under the conditions of illumination necessary for high-power microscopy. The ultra-violet photomicrographs reproduced by the courtesy of the late Professor Šatava are as near an approach to the ideal as is possible at present. Ultra-violet illumination cannot, however, be used for visual examination in the ordinary way, and it is not possible to see the detail revealed in these photomicrographs by white light.

For critical microscopy, including the detailed examination of bacteria, it is necessary to use objectives of the highest power and greatest aperture, that is a $\frac{1}{12}$-inch oil-immersion objective. It is generally only by such an objective that the gelatinous capsule round certain bacteria can be distinguished. An oil-immersion objective is, however, hardly practical for routine examination of yeast. A dry objective of lower power must generally be used. The magnification produced by an objective is found approximately by dividing 10 by its marked focal length when the tube is 10 inches or 250 mm. long. Thus, a $\frac{1}{12}$-, $\frac{1}{8}$- and $\frac{1}{6}$-inch objective will magnify, respectively, 120, 80 and 60 times. The final magnification of the observed image is then found by multiplying this by the power of the eyepiece, which may be × 4, × 6, × 10, etc., and by the tube length in mm. divided by 250. Personal vision will decide the best combination which, for rapid use, should be as low power as will adequately reveal all necessary detail. Some prefer a sixth objective with a × 10 eyepiece, which gives 600 magnifications. Others prefer an eighth objective with the same eyepiece, giving 800 magnifications. What these magnifications mean can be judged from the photomicrographs reproduced.

A pearl electric lamp, well shaded from the eyes, provides a useful illuminant. The flat side of the mirror should be used and the condenser nearly focussed by bringing almost to its topmost position. The working aperture of the iris diaphragm must be found by trial, but it should be as widely open as possible. An inexpert operator should carefully lower the microscope tube until it nearly touches the slide and focus upwards with the coarse and fine adjustments, to avoid damage to the cover glass or objective. Pressure may also destroy the yeast cells. The concave side of the mirror should not be used, and increased magnification must not be obtained by elongating the tube of the microscope. It should always be at the length for which the objective is corrected. Use of the concave side of the mirror, without a condenser, is very far from the ideal of critical illumination and may give very deceptive images. Modern prism binocular microscopes are preferred to monocular instruments by most workers. A mechanical stage is a refinement which greatly facilitates work, if accurately fitted and true in its movements. A plane stage with clips is much to be preferred to an inferior mechanical stage. A simple stand with high class lenses should be selected, but the stand must be rigid at the most comfortable inclination, with no slip or defect in the coarse and fine adjustments. These adjustments will rapidly be ruined if the microscope is lifted by any part other than the foot.

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CHAPTER XXXVII

BACTERIA

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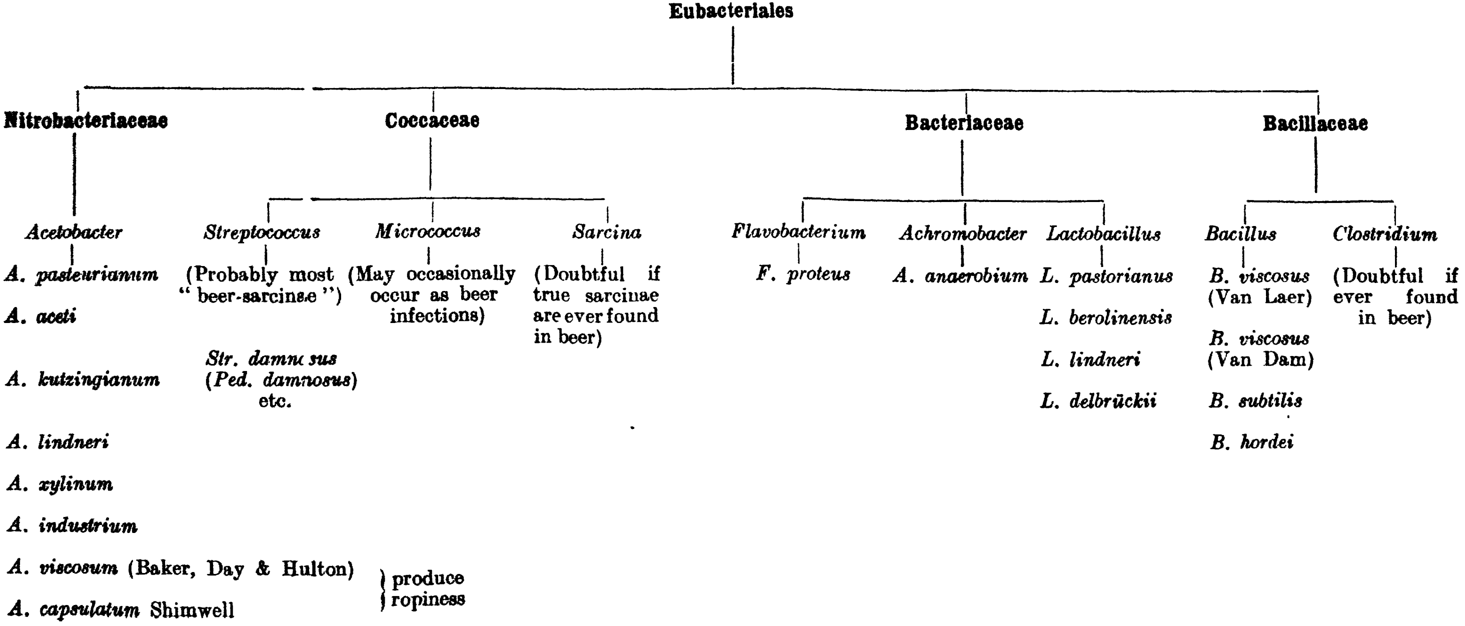
(658) Beer Bacteria.

Knowledge of the nature and identity of bacteria causing defects in beer has advanced very little since the publication of Pasteur's *Etudes sur la Bière* (1876). It is true that references to numerous bacteria, alleged to be capable of growing in beer with the production of various disorders, are to be found in the earlier literature after that date but, in many cases, the descriptions of the various species are too inadequate to permit of their identification to-day, whilst, in others, bacteria seem to have been recorded as beer species solely on account of their morphological resemblance to those seen in beer deposits, with little or no evidence that such bacteria had ever been detected in or isolated from beer itself. It is not intended, in this chapter, to repeat old descriptions of beer bacteria of doubtful authenticity, the names of many of which seem to have become established in the literature merely through reiteration. Furthermore, it has been thought unnecessary to multiply the number of species described by mention of certain forms that have been more recently isolated, but which, according to the available descriptions, appear to be of little or no significance to the brewer. The views expressed have, also, necessarily been based to a considerable extent on the writer's own opinions and experiences, since no review of brewing bacteriology has appeared for some time and recent investigations have been conspicuously lacking.

(659) Classification of Bacteria.

Most of the older classifications of bacteria were based almost entirely on morphology, but as the number of known species increased, this method became of decreasing utility, since many species of similar form differed widely in other respects. One of the results of this early method of classification was the accumulation of vast numbers of species with the generic names of *Bacterium* and *Bacillus*, both used almost indiscriminately to

TABLE 234.—ABRIDGED CLASSIFICATION OF BACTERIA MET WITH IN BREWING



- **Eubacteriales**
  - **Nitrobacteriaceae**
    - *Acetobacter*
      - *A. pasteurianum*
      - *A. aceti*
      - *A. kutzingianum*
      - *A. lindneri*
      - *A. xylinum*
      - *A. industrium*
      - *A. viscosum* (Baker, Day & Hulton) } produce ropiness
      - *A. capsulatum* Shimwell } produce ropiness
  - **Coccaceae**
    - *Streptococcus*
      - (Probably most "beer-sarcinae")
      - *Str. damnosus* (*Ped. damnosus*) etc.
    - *Micrococcus*
      - (May occasionally occur as beer infections)
    - *Sarcina*
      - (Doubtful if true sarcinae are ever found in beer)
  - **Bacteriaceae**
    - *Flavobacterium*
      - *F. proteus*
    - *Achromobacter*
      - *A. anaerobium*
    - *Lactobacillus*
      - *L. pastorianus*
      - *L. berolinensis*
      - *L. lindneri*
      - *L. delbrückii*
  - **Bacillaceae**
    - *Bacillus*
      - *B. viscosus* (Van Laer)
      - *B. viscosus* (Van Dam)
      - *B. subtilis*
      - *B. hordei*
    - *Clostridium*
      - (Doubtful if ever found in beer)

denote rod-shaped organisms and, thus, eventually losing almost all descriptive significance.

Attempts have been made during recent years to overcome some of the difficulties. Thus, the Society of American Bacteriologists has devised a classification in which bacteria are grouped, as far as possible, according to more natural affinities. The gigantic genus *Bacterium* has disappeared and the name *Bacillus* is only applied to aerobic, spore-forming species. The new classification, although not necessarily perfect or final, is a considerable improvement on the chaos which previously existed, and there is possibly little doubt that, with further modifications, it may eventually be adopted. The following is an abridged account of it, in so far as it affects beer bacteria. Details may be found in T. H. Bergey's *Manual of Determinative Bacteriology*, 1934, in which most well-defined types of bacteria are classified according to the new system.*

The Bacteria (Class *Schizomycetes*) are divided in the following orders : *Eubacteriales, Actinomycetales, Chlamydobacteriales, Thiobacteriales, Myxobacteriales, Spirochaetales.* All known bacteria causing defects in beer are found in *Eubacteriales,* which is divided in the following families : *Nitrobacteriaceae, Coccaceae, Spirillaceae, Bacteriaceae, Bacillaceae.*

No recorded beer species are found in *Spirillaceae,* which can therefore be omitted here. The other families are divided in *tribes* and the tribes in *genera.* As the number of tribes and genera is very large, and the number of known beer species is small, *tribes* have been entirely omitted in Table 234, whilst only those *genera* of importance in brewing technology have been included.

(660) Action of Hop Antiseptic on Bacteria.

Until very recently hopped wort was regarded as almost immune from attack by the majority of bacteria. It was believed that owing to the high antiseptic potency of humulon and lupulon only a few exceptionally resistant species could develop in their presence. It has now been found, however (Shimwell, 1937),[1] that many (and possibly all) Gram-negative bacteria (Section 672) are comparatively indifferent to or even stimulated by the hop rates normally used in brewing practice, and that hopped wort, far from being immune from attack, is actually more favourable to the growth of those very putrefactive bacteria which were previously thought to be particularly sensitive to the antiseptic principles of the hop.

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* Since going to press a 5th Edition of this Manual has been published in which considerable modification has been made in certain sections of the general bacteriological classification. For example, the genus *Streptococcus* is now included together with the genus *Lactobacillus* in a new family *Lactobacteriaceae,* thus indicating its true nature as a group of lactic acid bacteria. Its previous inclusion in the family *Coccaceae* was based solely on morphological criteria.

It is too early to speculate on the precise significance of this discovery on the relation between hop antiseptic and the bacterial flora of *beer.* Beer is a very complex mixture of nutrients and antiseptics and, owing to its low p_H, its bacterial flora must of necessity be fairly acid-tolerant. Many of the acid-tolerant bacteria happen to be Gram-positive (e.g., the *Lactobacilli*). Many Gram-positives however (*L. bulgaricus, L. delbrückii, L. plantarum* for example) are too hop-sensitive to grow in beer, whilst many Gram-negatives, although hop-tolerant, are too acid-sensitive to be found therein. Much more indeed requires to be known on the nature of individual beer species of bacteria before any generalisations can be indulged in. With regard to hopped *wort*, however, there seems no doubt that, contrary to previous beliefs, it is not protected from most common putrefactive bacteria by virtue of its hop antiseptic, since many of these organisms are Gram-negative.

In reading the following pages of this chapter, therefore, the Gram-staining reaction of each bacterial species discussed should be carefully kept in mind. It will be noted, for example, that when a Gram-negative species happens at the same time to be highly acid-tolerant (e.g., *Acetobacter capsulatum*), its development in the brewery cannot be prevented by an increase in hop rate. Some Gram-positive species, on the other hand, although innately hop-sensitive, are able to become acclimatised to quite high hop rates, e.g., *L. pastorianus* ("*Saccharobacillus*").

BEER BACTERIA

(661) Beer Defects of Bacterial Origin.

Among the chief bacterial disorders affecting beer are (1) acidity due to acetic acid bacteria, (2) acidity due to lactic acid bacteria, (3) ropiness and (4) the so-called "sarcina sickness." In addition to these defects, other changes influencing flavour and aroma may be brought about by other bacteria, including those Gram-negative species which are able to grow in wort, but do not survive the primary fermentation. Apart from the action of *Flavobacterium proteus*, little is known at present of the latter. Unhopped beer wort is, however, an excellent pabulum for many saprophytic bacteria and the fact that comparatively few species appear to be capable of growing in beer is probably due to its low p_H and the presence of alcohol and hop extractives. The various species of beer bacteria are grouped in the following Sections according to the type of defect they produce.

(662) Acetic Acid Bacteria (Genus *Acetobacter*).

The acidification of beer by acetic acid bacteria may not perhaps be regarded at the present time as a very great danger. These organisms require access to free oxygen ; serious souring, therefore, cannot take place unless the beer is unduly exposed to the air. Wooden vessels and accumulations of beer scale were a prolific source of infection in days gone by, but the greater cleanliness of modern breweries minimises this danger. Beer stored in casks for long periods for maturing may develop some acetic acidity and the vinegary smell of returned empties is also often partly due to these organisms.

A number of species have been isolated from beer and beer wort. *Acetobacter pasteurianum*, *A. aceti*, *A. kützingianum*, *A. lindneri*, *A. acetosum*, *A. xylinum*, *A. oxydans*, and *A. industrium* are all species stated to be capable of acidifying beer. In addition, two species of *Acetobacter* are capable of turning beer ropy. These are *A. viscosum* (Baker, Day and Hulton[2]) and *A. capsulatum* (a new species recently described by the writer[3]). These two species will be described in the section dealing with ropiness.

Most of the *Acetobacter* species mentioned above are fairly similar as regards morphology, being short, stumpy, Gram-negative rods, frequently united in pairs and chains ; many species are, however, highly pleomorphic, developing long swollen filaments under unfavourable conditions of cultivation. *Acetobacter* species can be detected in the brewery whenever beer or yeast is allowed to remain in contact with the air for any length of time. Their presence in many pitching yeasts can be demonstrated biologically, their aerobic nature preventing serious trouble arising when the yeasts are used in the brewery.

Although the conversion of ethyl alcohol to acetic acid is an aerobic process involving, under brewery conditions, access to air, some of these bacteria have also been found to possess anaerobic and fermentative functions under certain conditions. Thus *Acetobacter ascendens* and certain other species form alcohol and carbon-dioxide from glucose under anaerobic conditions (Neuberg and Simon, 1928[4]). Under aerobic conditions glucose is most commonly converted to gluconic acid by *Acetobacter* species, but a large number of different products have been recorded by different workers, including 6-aldehydo-gluconic acid, 5-keto-gluconic acid, succinic acid, lactic acid, glycollic acid, acetic acid, and formic acid. Since beer contains not only alcohol, but often also glucose, fructose, sucrose and maltose, the different changes which might take place under different conditions of aerobiosis and anaerobiosis are very complex. So far, however, the possibilities of the anaerobic development of acetic acid bacteria in beer has not been investigated. As far as the writer's experience goes, exclusion of air from beer prevents any serious damage by these organisms.

(663) Lactic Acid Rod Bacteria (Genus *Lactobacillus*).

The members of this genus are all Gram-positive, non spore-forming rods, usually regarded as micro-aerophilic. Their chief characteristic is their ability to convert carbohydrates almost solely to lactic acid, although some species produce not inconsiderable proportions of other products, such as acetic acid and alcohol. The name " *Lactobacillus* " is, perhaps, not too well chosen for this group. The ending "*-bacillus*" suggests spore formation which is, however, characteristically absent.

The genus contains several species of interest in brewing, notably :

L. pastorianus (*Saccharobacillus pastorianus* Van Laer).[5]
L. delbrückii (*B. delbrückii*).
L. bulgaricus (*B. bulgaricus*).

Lactobacillus pastorianus

Synonyms : Bacille des bières tournées (Pasteur)
Saccharobacillus pastorianus Van Laer.

This organism is the most commonly occurring bacterium in top fermentation breweries. Beers attacked by it exhibit a lustrous silky turbidity when agitated, together with a varying degree of acidity. This " silkiness " has usually been regarded as characteristic of *L. pastorianus*, but a similar phenomenon is produced by any long rod bacterium growing in liquid media. It was first isolated in 1892 by Van Laer. The cultural characters of the writer's strain[6] on solid media are depicted in Fig. 214, A, B and C. *L. pastorianus* is usually regarded as a long rod, averaging say 5 to 10 μ and producing pairs of cells united at an obtuse angle (see Fig. 215, A, which depicts a typical deposit of " turned " beer). It can, however, also exist as short stumpy rods, a matter of some importance in the microscopic examination of yeast for bacterial contamination (see *F. proteus*, Section 671).

The combating of " *Saccharobacillus* " infection in the brewery is best effected, as far as present knowledge goes, by as high a hop rate and as low an initial p_H at racking as possible and by keeping the residual *fermentable* extract at a minimum, since it is from residual sugar that the acid is formed. (N.B.—A high percentage attenuation does not necessarily indicate absence of fermentable extract.) The infection itself may be reduced by scrupulous cleanliness in the brewery and by cleansing the yeast by Horace Brown's[7] tartaric acid method (Section 576) if pure yeast culture is not used. Although progressively inhibited by increasing concentrations of hop antiseptic, *L. pastorianus* can become acclimatised to comparatively high concentrations and has been found capable of adapting itself to growth in beers with as much as 4½ lb. of hops per barrel. When cultivated in unhopped or weakly hopped beer for some time, it gradually loses its resistance to hops, and may then fail to develop in a beer similar to the one from which it was originally isolated.

The organism is tolerant of high concentrations of hydrogen ions when once it has commenced growth in a beer, but finds commencement of growth increasingly difficult at progressively lower p_H values. The buffer concentration of a beer would therefore appear to be of less importance in practice than its initial p_H, except in so far as buffer concentration serves to act as a component in determining the initial hydrogen ion concentration. It is often stated that "*Saccharobacillus*" in some forms is very resistant to heat. The writer has found no evidence of this. The organism is probably easily killed at the lowest temperatures used in pasteurising beer (say 130° F.). Belief in its heat-resisting powers may be due to its earlier confusion with *Bacillus subtilis.*

The original source of infection in a brewery is not easy to determine. Probably, in the first place, the infection comes from the air and then accumulates in the plant and the pitching yeast. *L. pastorianus* infection is, indeed, very difficult to eradicate permanently and constant vigilance is necessary if one is periodically troubled with this organism.

L. berolinensis (*Saccharobacillus pastorianus* var. *berolinensis* Henneberg) and *L. lindneri* (*B. lindneri* Henneberg) are organisms very similar to *L. pastorianus* and may indeed be varieties of the latter; they cause the same type of trouble and were isolated from Berlin Weiss-Bier and light lager respectively. The three species differ slightly in their temperature relations and their sugar fermentations, but seem otherwise not very dissimilar.

Lactobacillus delbrückii

Synonym: *Bacillus delbrückii* Leichman
Probable synonyms: *Bact. acidificans longissimum* Lafar
Thermobacterium cereale Orla-Jensen.

This is not a beer species but is used in breweries and distilleries for the artificial acidification of the mash (Section 457), its growth and acid production at high temperatures inhibiting most harmful bacteria. Further interest to brewers lies in the fact that it has been employed in the biological estimation of the preservative value of hops. It will not develop in quite lightly hopped beer, even at its optimum temperature (115°–117° F. according to Henneberg).

Lactobacillus bulgaricus

Synonym: *Bacillus bulgaricus* Grigoroff.

This bacterium is of milk origin and is also only of interest to brewers through its employment in hop tests. Since neither this species nor the preceding will grow in beer, their use in this connection is possibly open to criticism.

Some of the older textbooks lay stress on the importance of a " *Bacterium lactis* " as one of the chief causes of acidity in beer, but no references to the literature are quoted and the writer has been unable to find any. " *Lactis* " is, however, a very popular specific name in old brewing bacteriological nomenclature, so there appears little chance of identifying the beer organisms referred to, since no description is available. There seems no doubt that beer deposits do frequently contain short rod bacteria and that their presence is often associated with acidity. Possibly in some cases these short rods are a short form of *L. pastorianus*, but the possibility of the existence of other common acid-producing beer-species cannot be excluded.

(664) Ropiness.

Although much has been written on the subject of ropiness in beer, the sum total of precise knowledge obtained as the result of a study of the literature does not amount to very much, but the general consensus of practical brewing opinion in Great Britain seems to be that a species of coccus or " sarcina " is nearly always to be found in samples of ropy beer. Brown and Morris,[8] in 1895, isolated a coccus from ropy beer and traced the source of infection to a local pork butcher's shop, but unfortunately they did not study the organism except from the immediately practical point of view; it is consequently unnamed and unidentifiable to-day. Kayser[9] (1913) found a coccus (unnamed) to be responsible for ropiness in beers brewed in Normandy, whilst Schönfeld and Himmelfarb[10] (1912) also described a slime-producing coccus. Neither of these organisms however have, so far, been identified in ropy British beers.

On the Continent much ropiness seems to have been due to rod-shaped bacteria, cocci or so-called "sarcina" being more rarely associated with this type of trouble. H. van Laer[11] (1889 and 1900) has described three types of *Bacillus viscosus* (*I*, *II* and *bruxellensis*) but these have never been found in British beers. Van Dam[12] (1896) reported a *Bacillus viscosus III* from English beer, but this also finds no further mention since its first isolation. In 1912, however, the results of an important investigation by Baker, Day and Hulton[2] were published. Ropy beers from different sources in the British Isles were examined and the organism responsible isolated. The bacterium was found to be of the same species in each case and was given the name *Bacterium aceti viscosum* which is now amended to the more modern binomial *Acetobacter viscosum*, since the organism is a member of the acetic acid bacteria and is closely related to *A. aceti*, and the other well-known brewery species.

Recently another *Acetobacter* species capable of causing ropiness in beer has been described by the writer[3] and has been named *Acetobacter capsulatum*. Although these two species show considerable cultural and physiological differences, the most important difference from the purely practical viewpoint lies in their temperature relations, *A. viscosum* growing best at 68° F., *A. capsulatum* at 90° F. Both species are aerobic, so that exclusion of air from the beer is the best method of combating infection by these organisms. Neither species was affected by increases in hop rate up to 5 lb. per barrel; in fact *A. capsulatum* actually grew better at this figure. (It is Gram-negative.) The latter organism was equally indifferent to high hydrogen ion concentration, growing vigorously in a beer adjusted to p_H 3·4 with lactic acid.

The production of ropiness by *A. capsulatum* was found to be due to the formation of gelatinous capsules (see Fig. 216, C, D and E). No such capsules, however, were detected by Baker, Day and Hulton in the case of their *A. viscosum*. A notable feature of *A. capsulatum* was its ability to adopt a coccus form (see Fig. 216, B), a point possibly not without significance in view of the widespread belief in the presence of cocci in ropy beer. Chains were sometimes but rarely produced (Fig. 216, E). All beers were found to be not equally susceptible to the production of ropiness by these two species. In the case of *A. capsulatum*, this was found to be solely due to small variations in alcohol concentration. Although able to convert alcohol into acetic acid, increasing concentrations of alcohol increasingly delayed the commencement of growth, a difference of only 0·5 per cent. by volume in the region of 6 per cent. postponing ropiness by several weeks at ordinary temperatures. *A. capsulatum* was found to be capable

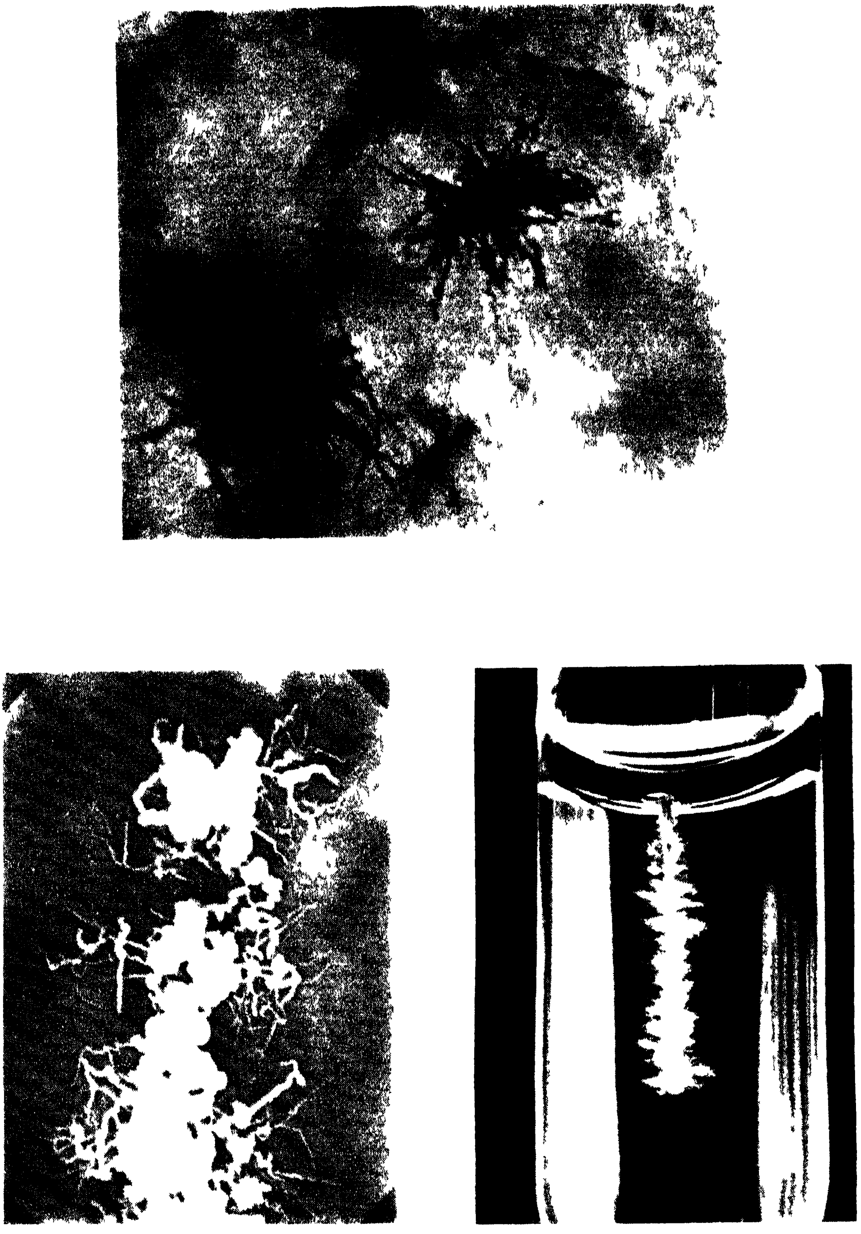

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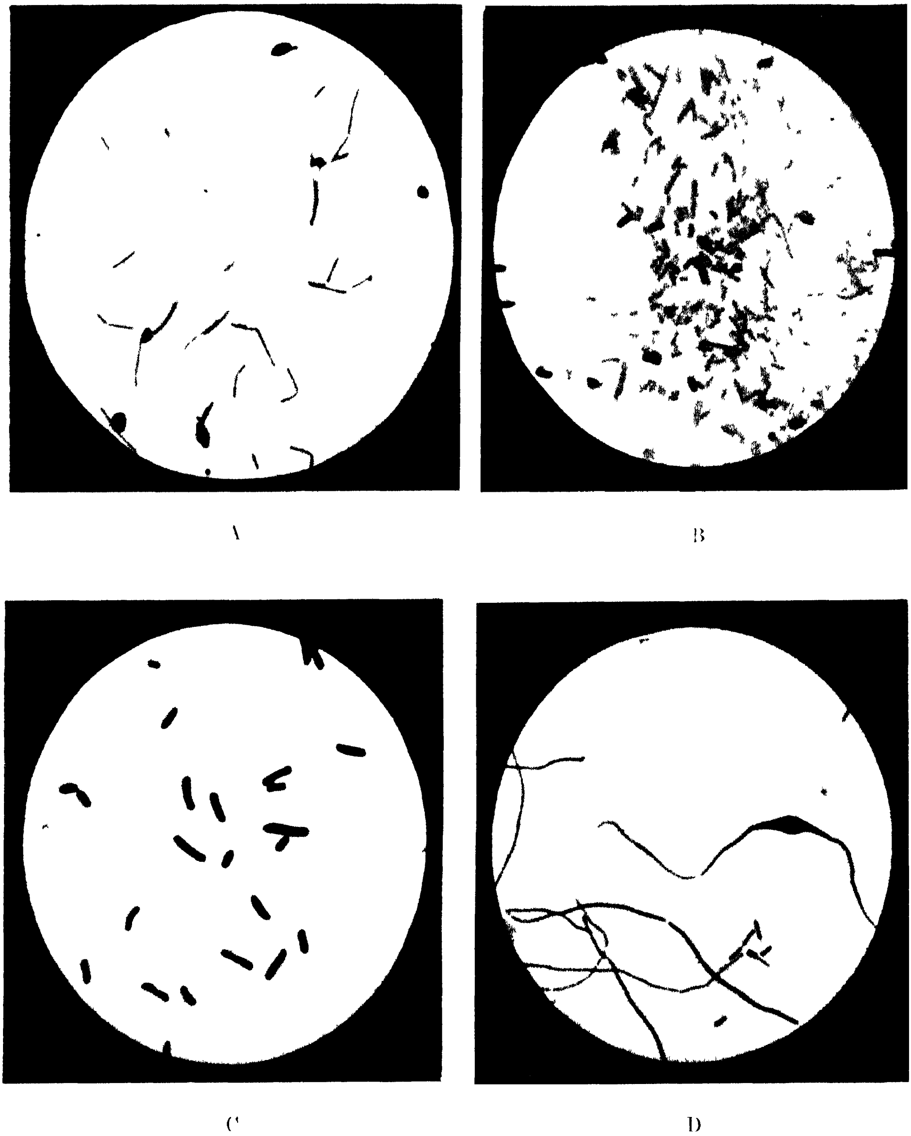
Fig. 214 *Lactobacillus pastorianus*

A Colonies in Wort agar × 60 transmitted light

B Streak culture on Wort gelatin × 15

C Stab culture in Wort gelatin × 2



A B C D

Photos Shimwell and Kirkpatrick

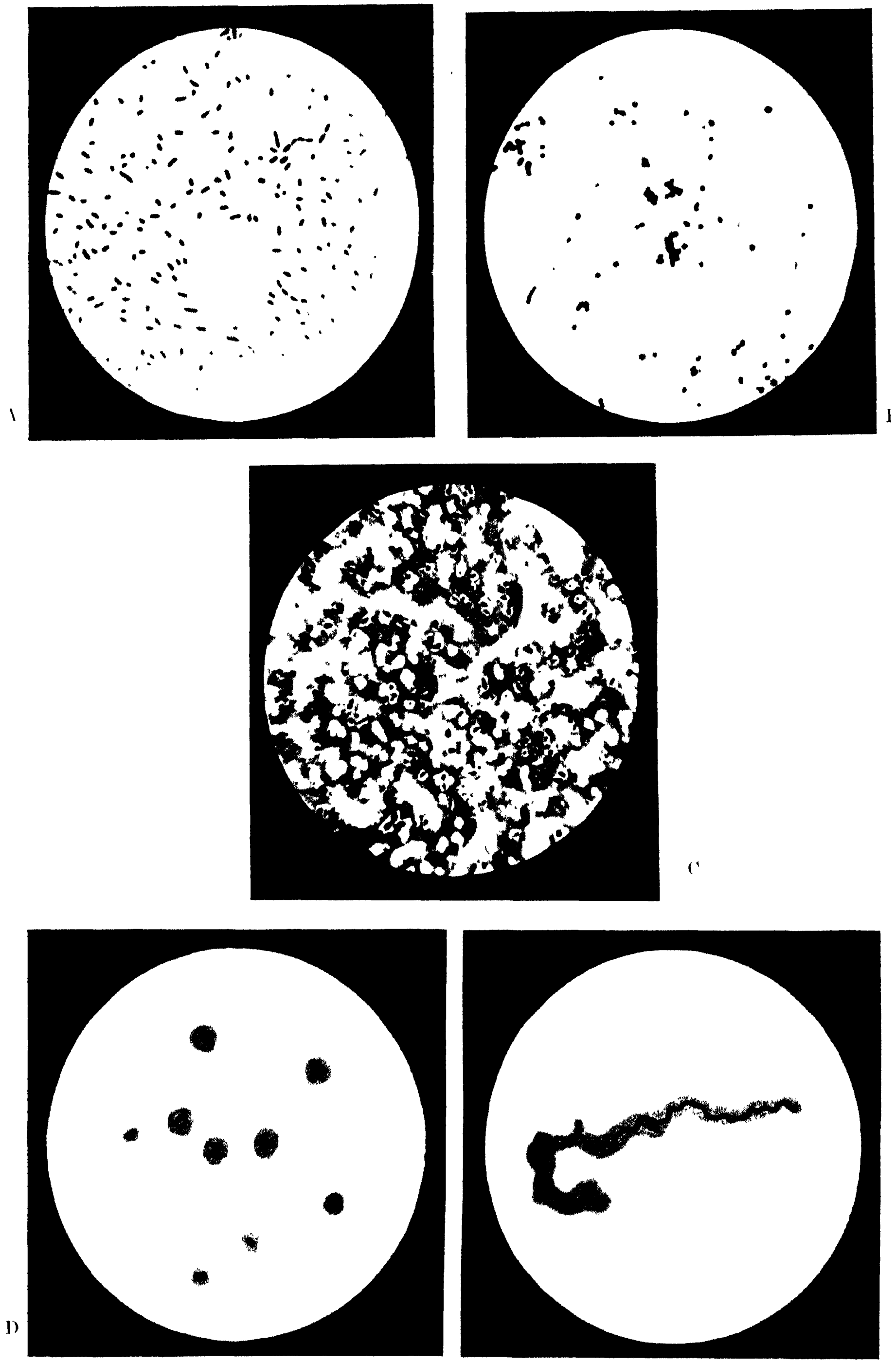
FIG. 215 *Lactobacillus pastorianus* and *Flavobacterium proteus*

A. *L. pastorianus* × 450 'Turned' beer deposit

B. *L. pastorianus* and *F. proteus* × 1000 Gram stained

C.—*F. proteus* × 1200 Rods from wort agar

D. *F. proteus* × 600 Abnormal forms



Photos : Shimwell and Kirkpatrick

FIG. 216. *Acetobacter capsulatum* × 1000

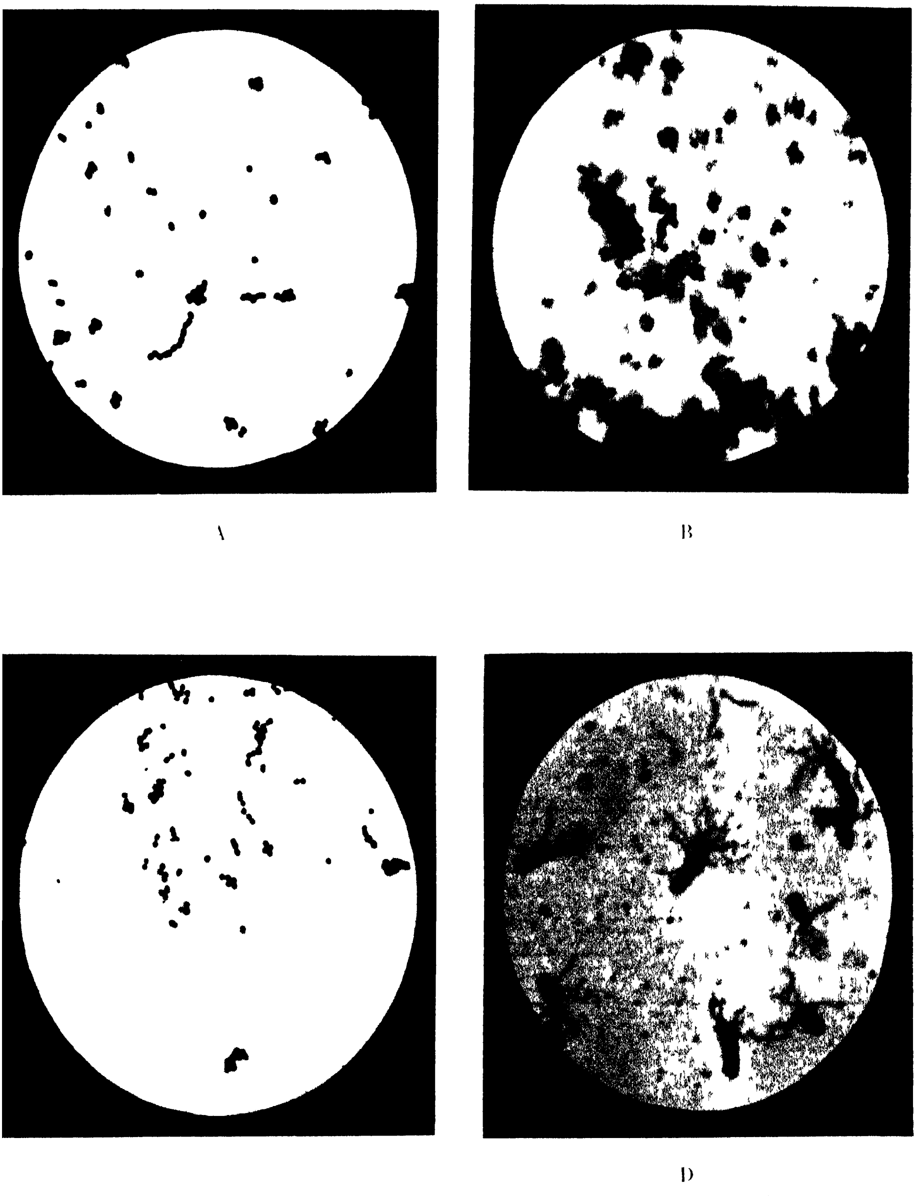
A.—Rod form (from broth)

B. Coccoid form (from beer) carbol-crystal violet stain

C.- Zooglea, Capsule stain

D.—Capsulated groups, Capsule stain

E.—Capsulated chain, Capsule stain



Photos A, B and C Shimwell and Kirkpatrick
Photo D J L Shimwell

FIG 217 COCCI AND *Achromobacter anaerobium*

A—*Streptococcus damnosus* (*Pediococcus damnosus*) × 1000

B Rope producing *Streptococcus* ("*Sarcina* or *pediococcus*") beer culture, showing slime faintly stained × 1000

C—*Streptococcus bovis* × 1000

D—*A anaerobium* showing polar flagella × 1425 of existing in pitching yeast and also of growing vigorously on the yeast cap left on the beer after skimming. Although aerobic, sufficient air was often found present in beer after bottling to enable the organism to bring about ropiness.

In addition to the production of ropiness in beer by the organisms mentioned above, it is not impossible that it may sometimes be caused in beer by bacteria which are normally not slime-producers. Certain species of bacteria such as *Aerobacter hibernicus*, *Streptococcus lactis*, and even *Lactobacillus bulgaricus* are sometimes found to possess slime production as a temporary character under certain conditions.

The yeast-like mould *Dematium pullulans* (Section 655) was found by Lindner (1888) to be capable of causing ropiness in wort, but this organism is not usually regarded as of much importance in the brewery. In the writer's opinion, however, it is of more common occurrence there than is generally supposed, several cases of ropiness in hopped wort having been encountered in which *Dematium pullulans* or a closely related mould was found to be the causal organism. There appears to be no record of its causing trouble in *beer.*

The production of ropiness by so-called " beer-sarcinae " is referred to in Section 670.

(665) Spore-forming Bacteria (Genera *Bacillus* and *Clostridium*).

Great stress is frequently laid on the necessity for a good boiling of the wort in order to kill any bacteria which may be present, particularly spore-forming species. The writer is convinced that the alleged danger from spore-forming bacteria is exaggerated. Probably very few spore-formers are capable of growing in hopped beer and all non-sporing species would be killed at temperatures far below the temperature of boiling wort. The widespread belief in the heat resistance of beer-bacteria is probably a relic of the days (not so long ago) when *B. subtilis*, " *B. ulna*," etc., were regarded as common beer organisms. Bacterial spores might conceivably survive the boiling of wort (despite its low p_H and high hop antiseptic content) and, perhaps, pasteurisation of the finished beer. The fact that bacterial trouble rarely, if ever, occurs in pasteurised beer, however, is an indication that such survivors (if any) are of species incapable of growing in that medium.

The genus *Bacillus* contains all aerobic species of spore-forming bacteria and the genus *Clostridium* all anaerobic forms, which are probably of comparatively little interest to the brewer, although of more importance in distilling. Prior to, and even long after the work of Van Laer on " *Saccharobacillus pastorianus* " (*L. pastorianus*), ***Bacillus subtilis*** was regarded as the principal cause of the "turning" of beer, this belief being apparently based solely on the size and shape of the organism. This belief has been long exploded, but according to N. Van Laer,[13] *B. subtilis* and *B. hordei* (a species isolated from barley) can, to a limited extent, grow in beer with the production of objectionable harsh or woody flavours. Curiously, however, these bacteria were not found to grow in hopped wort. The spores of both these species are reported to have been killed by boiling in hopped wort for 30 minutes. Other beer bacteria which have been reported as forming spores are *Bacillus viscosus I* and *II* (Van Laer) and *Bacillus viscosus III* Van Dam, which will be found referred to under " Ropiness."

It should be noted that the use of " *Bacillus* " as a generic name in past brewing literature has little or no descriptive meaning except "a rod," and should not be taken to denote a spore-former unless such is definitely stated in the original literature. As nearly all spore-forming bacteria are Gram-positive, it is probable that they are hop-sensitive. The writer has confirmed this in a number of cases. The present cultures of *Bacterium X* (A. Brown) and *Bacterium C* (Chapman) maintained at the Lister Institute are not beer bacteria,[14] but similar to if not indentical with *Bacillus cereus* Frankland.

(666) Achromobacter anaerobium.

In 1937 a new type of bacterial infection in beer came to light, caused by a motile, anaerobic, Gram-negative bacterium which has been given the name *Achromobacter anaerobium* (Shimwell).[15]

Infection by this organism combines the worst features of wild yeast and bacterial contamination since, as the bacterium is Gram-negative, it is indifferent to the action of hop antiseptic, and being also anaerobic, the exclusion of air only serves to encourage its development. It grows with extraordinary rapidity in primed beer, which it is capable of rendering unsaleable during warm weather in two or three days after racking. The beer becomes densely turbid with a silky turbidity similar to that produced by *L. pastorianus*, but of somewhat less lustre. The flavour of the beer is impaired and the " nose " becomes unpleasant —a slight H_2S " stench " combined with an odour of apples. Abundant gas-formation also takes place.

This bacterium is tolerant of high hydrogen ion concentrations and will grow rapidly in highly hopped beer even at p_H values below 4·0. It has, however, an Achilles' heel by which it may be combated in some cases—it develops only in *primed* beer. It does not attack maltose, nor apparently " malto-dextrins " and shows little or no development unless the beer contains glucose or fructose. Cane sugar is attacked only slightly or not at all. If this bacterium is present in beer, however, priming with glucose or invert (or with impure cane sugar) will cause a very rapid development of the trouble. Lactose is not attacked at all. The action of this species on glucose is, unlike that of most bacteria, very similar to that of a yeast. The sugar is converted into almost quantitative proportions of alcohol and carbon dioxide, little or no acid being produced.

A. anaerobium grows best at about 85° F., so that hot weather is likely to accentuate its development. Even at cooler temperatures, however, its growth in air-free beer is more rapid than that of any other known beer species. In appearance, it is a short or medium-length plump rod (2 to $10\mu \times 1$ to $1{\cdot}5\mu$), actively motile when young, but in beer it becomes non-motile after a time and often appears in the form of star-shaped clusters. It does not form spores and is readily killed by exposure to a temperature of 140° F. for five minutes or more.

For details of the distinguishing characters of *A. anaerobium*, reference should be made to the writer's original communication to the Institute of Brewing (loc. cit). It should be borne in mind that although the strain described ferments only glucose and fructose, sucrose-, lactose- and maltose-fermenting strains may exist. If this organism is encountered in a brewery, every effort should be made to eliminate every possible source of infection, as it is an extremely dangerous species and very difficult to combat in practice.

SARCINA

(667) Types of Cocci.

No aspect of brewing bacteriology has received so much attention with perhaps so little result as the study of the so-called "beer-sarcinae." The literature of "sarcina-sickness" would, in fact, fill several large volumes and it is impossible to do more than briefly to summarise it in the course of this present chapter. The spherical bacteria are usually divided in three main types, (1) the sarcinae, which divide in three planes, yielding symmetrical cubical blocks or packets of spheres, (2) the micrococci, which divide in two planes giving pairs, fours and irregular clumps, and (3) the streptococci, which divide usually in one plane yielding pairs and chains of varying length. The cocci responsible for the so-called "sarcina-sickness" were at first believed to belong to the first of these groups, and were named "sarcinae" by Hansen but later observers have been almost unanimous in asserting that the beer "sarcinae" are not sarcinae at all but "pediococci," the latter being merely a popular term apparently synonymous with "micrococci," which is the usual scientific name for the "mass-forming cocci" which divide in two planes yielding the tetrads alleged to be characteristic of beer-types.

(668) Micrococci.

The micrococci are exceedingly common in nature and dwell in numerous habitats, having been isolated from such varied sources as air, water, skin, milk, dung, decaying animal matter, etc., etc. A large number of species have been described, but according to Hucker[16] they can be collected in nineteen different species, of which seven give a white or colourless growth on agar, five yield a yellow pigment, two an orange pigment, whilst the remaining five species give a characteristic red colour. The red types are classified in Bergey's *Manual of Determinative Bacteriology* as a separate genus known as *Rhodococcus*, whilst parasitic species of any colour are classed therein as *Staphylococcus*. All these types, however, are very similar, and are characterised by the formation of circular glistening colonies on solid media and the production of a more or less luxuriant surface growth on nutrient agar (peptone and meat extract).

It is natural to think that these *Micrococcus* species, so common in the air of breweries, in malt dust, in water and in many of the materials associated with brewing, should be the source of sarcina infection, causing "sarcina-sickness" in beer, but such a general connection has never authentically been established. Except in a few doubtful instances, it has been found that the micrococci and the "beer-sarcinae" were of entirely different types, incapable of mutual transformation the one into the other. The writer has personally attempted to grow all Hucker's nineteen *Micrococcus* species in beer, but in not a single instance has any sign of growth been detectable, whilst species of "beer-sarcinae" isolated from beer grew readily in beer with the production of the typical symptoms of "sarcina-sickness," i.e. more or less turbidity, more or less acidity, and the characteristic honey-like odour usually associated throughout the literature with "sarcina-infected" beer.

(669) Pediococci.

In course of time many hundreds of strains of so-called " sarcina " have been isolated from beer by different investigators, and have been given various names, according to the differences they have exhibited when grown in beer. *Pediococcus sarcinaeformis*, *Pediococcus acidulefaciens*, *Pediococcus odoris mellisimilis* are notable as referring to morphology, acid production and honey-aroma, respectively, whilst Claussen (1904 *et seq*)[17] found that the majority of these cocci fell into two types, which he called *Pediococcus damnosus* and *P. perniciosus* respectively, the former growing as a deposit, and the latter remaining suspended in beer, forming a haze. R. H. Mees[18] was the first to study the " sarcina " problem from a scientific rather than a technical point of view, and, collecting species of " beer-sarcinae " from beer, " pediococci " from horse urine, and micrococci from other sources, came to the conclusion that the " beer-sarcinae," together with one or two cocci from other sources, belonged to the true lactic acid bacteria, but differed from any types previously discovered. He proposed to create a new genus for their inclusion under the name *Pediococcus* (used this time, however, in a true generic sense rather than as a common or technical name, as had previously been the case). *Pediococcus damnosus* Claussen was regarded as the type species, other strains as varieties of this.

(670) Streptococci.

In 1938 Shimwell and Kirkpatrick[19] reopened the question of the true nature of the " beer-sarcinae " and, as a result of a study of representative strains of these organisms from widely different sources, together with a critical review of the literature, came to the conclusion that the beer-cocci were neither sarcinae, " pediococci " nor micrococci, but belong to the plant division of the genus *Streptococcus*. The morphological barrier, which may have prevented previous consideration of this genus for the inclusion of the " beer-sarcinae," was found to be far from insurmountable, for the beer-cocci can produce chains of cells and the streptococci can produce apparent tetrads due to the adherence of two diads or pairs. Fig. 217, A and B, show this overlapping of morphology with *Pediococcus* and *Streptococcus* types.

Culturally and physiologically, the characters of the beer-cocci correspond closely with those of the genus *Streptococcus*, not the least important coincidence being that of " aroma "-production. Certain species of *Streptococcus* are of great importance in dairying; they are responsible for the characteristic odour of fresh butter, which is due to the production of diacetyl ($CH_3CO—CO·CH_3$), and are technically known as "the aroma-streptococci." Shimwell and Kirkpatrick[19] have shown that "sarcina-odour" is also due to the production of diacetyl, which, mingling with the basic odour of the beer, produces the honey-like smell characteristic of "sarcina-sickness." These authors consider that the "beer-sarcinae" are closely related to the aroma-streptococci and that those so far studied can probably be included in a single species, for which the name *Streptococcus damnosus* would appear to be valid. Varieties of this species exist, however, notably a slime-producing type, for which the name *Str. damnosus* var. *viscosus* is suggested.

Streptococcus damnosus is described as Gram-positive, non-motile, non-sporing, catalase-negative and as producing usually the inactive form of lactic acid. It forms pairs, tetrads and chains of cells (largely depending on the medium), but its common form in beer often takes that of irregular, three-dimensional clumps, sometimes difficult to distinguish from amorphous matter. It is hop-sensitive, but may acquire considerable resistance to hop antiseptic and develop in even the most heavily-hopped beers, particularly the slime-producing type. Some strains are very acid-tolerant, and may carry the p_H down to p_H 3·6 even in hopped beer.

One variety of *Str. damnosus* produces ropiness in beers but this property is a very variable one and may be lost or acquired without apparent cause. It has been found, however, that absence of air and saturation with CO_2 are factors contributing to slime production. Slimy strains may fail to produce ropiness if air is present or CO_2 absent. Under such conditions only the ordinary symptoms of "sarcina-sickness" may be exhibited. The ropiness seems to be due to the production of capsules which dissolve or disperse into the beer, with consequent increase in viscidity. Brewers have long believed that a species of coccus was often responsible for ropiness in British beers; the work referred to supports this contention.

It is important to note that beer streptococci will not usually grow at high temperatures. They grow best at about 75° F. or even lower and their development may be checked or even prevented entirely during the "forcing" test, if incubated above 80° F. A temperature of 85° may give an unduly optimistic stability prediction in the case of these organisms.

From the literature it appears that the type of coccus referred to in this section is that most commonly responsible for "sarcina-sickness," but there is also evidence that aerobic, catalase-positive

Micrococcus species are occasionally able to develop in beer. As an example of this type of infection in British beers, the investigation by Heron[21] (1923) is of particular interest. The organism was aerobic, probably catalase-positive, and produced yellowish green colonies (an unusual colour for this type of bacterium). It produced intense turbidity in beer, but no great alteration in flavour. It was hop-sensitive and could be checked by an increase in hop-rate. Other cocci producing pigment occasionally find mention in the literature, notably that isolated by Brown and Morris in 1895.[8]

BACTERIAL INFECTION OF PITCHING YEAST

(671) Flavobacterium proteus.

Very many samples of top fermentation pitching yeast are found to contain a varying percentage of rod bacteria, generally referred to by brewers as "the short fat rods." These short rods are usually regarded as comparatively harmless, since they rarely seem to develop in a finished beer. The nature of these common yeast bacteria has been studied by Shimwell and Grimes[22] (1936). The predominating bacteria were isolated from samples of yeast from different sources, and were found to consist of the same species in each case, the organism being given the name *Flavobacterium proteus* on account of its marked pleomorphism.

F. proteus is a Gram-negative, non-sporing, non-motile facultative anaerobe. It is solely a wort organism, as it was found incapable of development at the p_H of beer (4·2). The products of its action on wort, both hopped and unhopped, were found to include a small percentage of volatile and non-volatile acid, a trace of ethyl alcohol and a considerable quantity of gas consisting of hydrogen and carbon dioxide. Its action in the brewery seems to be limited to the primary fermentation, before the p_H falls to an inhibiting value. *F. proteus* produces a pronounced aromatic odour and flavour in both hopped and unhopped wort, and it seems possible that an excessive infection of this organism might have some influence on the character of the resulting beers. The aroma produced is of a fruity type somewhat reminiscent of parsnips.

Some idea of the pleomorphism displayed by this bacterium may be gained from Fig. 215, C and D. The organism prefers a slightly acid medium (p_H 5) and tends to produce long involution forms in neutral or alkaline media (Fig. 215, D). The optimum temperature is 90° F. (32° C.) but quite a good growth takes place at room temperatures. The organism was found to reduce nitrates to nitrites, another point of possible practical significance in view of the alleged importance of nitrates and nitrites in yeast nutrition. The evolution of hydrogen during a brewery fermentation is also possibly not without importance.

(672) Detection of L. pastorianus in Pitching Yeast.

In view of the pleomorphism displayed by both *F. proteus* and *L. pastorianus*, and since both these species are capable of accumulating in pitching yeast, an ordinary microscopic examination of a yeast sample cannot with certainty determine the relative proportions of these two organisms. They can be distinguished however by taking advantage of the different reaction to Gram's stain. The following is the method found suitable by the writer.

(1) Separate the bacteria from a suspension of yeast in water by means of a centrifuge.

(2) Make a dense cover glass film of the bacterial deposit thus obtained. Dry and fix in a flame in the ordinary way.

(3) Stain with carbol gentian-violet (or crystal violet) for about 5 minutes. (1 part of a 10% solution of crystal violet in alcohol to 10 parts of 1% phenol solution.)

(4) Without washing, treat with Gram's iodine solution for 1 minute. (Iodine 1 grm., pot. iodide 2 grm., water 300 ml.)

(5) Without washing, decolorise in rectified spirit for two or three minutes.

(6) Wash in water, dry, and counterstain preferably with aqueous alcoholic Aurantia for 5 minutes, or with Safranin for a few seconds. (2·5% solution of Safranin in 95% ethyl alcohol, 1 part; distilled water, 10 parts.)

(7) Wash, dry and mount.

Any "*Saccharobacillus*" rods present will show up as a deep blue against a background of yellow or red *F. proteus* cells (Fig. 215, B). This test is, of course, not specific, but further confirmation of the presence of "*Saccharobacillus*" may be obtained by seeding a quantity of sterile, lightly hopped beer with a portion of the centrifuge deposit, whereupon "the turn" will be produced if "*Saccharobacillus*" is present. (At the time of writing *L. pastorianus* and its close cousins *L. berolinensis* and *L. lindneri* are the only species known to be able to cause the " turning " of beer.)

If any such infection is found to be existent in the yeast, the number of bacterial cells which may be introduced into the wort, when that yeast is used for pitching, may be far in excess of that which might gain access to the beer by direct aerial infection. The presence of one bacterium per hundred yeast cells, for example, means the introduction of over 100,000 bacteria into *each millilitre* of wort, if a pitching rate of 1 lb. per barrel is used.

GROWTH OF BACTERIA

(673) Phases in the Growth of Bacteria.

When a number of bacterial cells are introduced into a new medium, multiplication does not commence at once and proceed at a uniform rate, but progresses in a succession of well-defined phases, which have been summarised by Buchanan as follows :

(1) *An Initial Stationary Phase* during which no multiplication takes place.

(2) *A Lag Phase* during which the growth rate accelerates to a maximum.

(3) *The Logarithmic Growth Phase* during which the growth rate is constant, the number of bacteria increasing logarithmically with time.

(4) *The Negative Growth Acceleration Phase* in which the rate of increase of numbers slows down.

(5) *The Maximum Stationary Phase* in which the death rate equals the multiplication rate, and the number of living cells remains stationary.

(6) *The Accelerated Death Phase,* during which the bacteria die off at an increasing rate.

(7) *The Logarithmic Death Phase* during which the number of living cells diminishes in logarithmic order against time.

These growth phases, particularly the first two, are of the utmost importance in all problems affecting the " bacterial stability " of beer, but seem to have been almost completely ignored in brewing literature until very recently, although Walker noted their significance in connection with his log phase method of measuring antiseptic potency (Vol. I, Section 271). The existence of the initial stationary phase, for example, which is prolonged in some cases by the presence of such antiseptics as hops and alcohol, has particularly to be taken into account when " forcing tray " results are being studied. It is not safe to assume, for example, that, because a beer shows no signs of bacterial growth after say 10 days' incubation (or "forcing") at 80° F. it will not subsequently develop bacterial trouble if incubated longer than this period. A further 24 hours may result in an abundant development of bacteria. The commencement of growth of bacteria in beer may be delayed for quite long periods after infection, but when it does take place it may commence suddenly and perhaps proceed very rapidly, so that a beer which was perhaps regarded as perfectly " sound " may suddenly display unsuspected instability.

It is a common practical experience that the results of forcing tray tests are not always borne out by the behaviour of the beer in normal trade conditions, its stability being sometimes worse and sometimes better than that predicted from the forcing test. Variation in the duration of the stationary phases of the bacteria at the different temperatures employed in " forcing " and storage respectively may often be responsible for this phenomenon. *L. pastorianus*, particularly, can remain in the stationary phase in beer for long periods and then be capable of sudden rapid growth and acid production, providing the yeast present has not removed all the residual fermentable carbohydrates during this time.[6] The function of hop antiseptic during the primary fermentation may also partly be to postpone the development of Gram-positive bacteria until the culture yeast present has lowered the p_H to a point at which such bacteria cannot grow or can only grow with difficulty.

(674) The Attainment of Bacterial Stability in Beer.

There has always been a desire on the part of brewing technologists to discover some single means (apart from pasteurisation) by which beer could be rendered resistant to bacterial attack, and from time to time various theories as to the best method of attaining this object have held sway. For very many years " the Nitrogen Question " held the attention of brewers and " residual non-coagulable assimilable nitrogen " was regarded as the be-all and end-all of bacterial instability, which, despite the almost complete absence of evidence in support of this theory, and in face of considerable evidence to the contrary, was considered to be due to an excess of nitrogenous bacteria-food in the beer. It is not impossible that, in the case of some bacteria, variations in nitrogen concentration may not be without considerable influence on bacterial stability, but the tendency at the moment is largely to discount the exaggerated significance which has for so long been attributed to " excess residual nitrogen " in beer. In this connection the work of Hulton and Baker,[23] Hopkins and Fraser,[24] and Shimwell[6] may be referred to.

Following the " Nitrogen Question," the discovery of the influence of the hydrogen ion concentration (as distinct from the titratable acidity) on the growth of bacteria was welcomed as a possible solution of the brewers' problem, but here again perhaps too much was expected of p_H control, and a revival of belief in the efficacy of hop antiseptic took place, although the two factors are to a certain extent considered together in view of some evidence that the potency of hop antiseptic may be dependent on the hydrogen ion concentration. Recently, however, doubt has been expressed as to whether the antiseptic power of hops is as great as was previously supposed, and attention seems now to be turning to the question of the effect of the oxidation-reduction potential of beer on " bacterial stability."

This tendency to seek the discovery of some single factor, the control of which will be effective towards all bacteria at once, has been one of the features of brewing technology during several decades and is all the more surprising since little has been known as to the nature and identity of the bacteria towards which such expedients would be directed in practice. Recent work tends to show, however, that the physiology of individual beer species varies so widely that it would be difficult, if not impossible, to discover any one factor, the control of which would be effective towards them all. Whilst an increase of hop rate and initial hydrogen ion concentration would be, to some extent, effective in checking *L. pastorianus*, for example, such measures would be quite ineffective against *A. capsulatum*, which is indifferent to variation in these factors.

In addition to the other precautions already indicated in this chapter, a high hop rate and a low initial p_H are possibly the best routine measures that can be taken against general bacterial infection, but it does not necessarily follow that trouble may not be experienced despite these precautions. If, under these conditions, bacterial infection is still experienced, a knowledge of the physiology of the particular organism responsible is necessary. Without such diagnosis, treatment can only be by a process of trial and error. Whilst brewing science has always of recent years been eager to adapt to its needs any new discovery in physics or chemistry likely to benefit "bacterial stability," it has been strangely diffident in its attitude to that equally important part of the question—the nature of the beer-bacteria themselves.

(675) Summary.

Although brewery bacteria were amongst the earliest micro-organisms described by Pasteur, their subsequent study has been greatly neglected for many years. There has arisen a tendency to regard all beer bacteria as similar in their reaction to their physical and chemical environment, with the result that empirical methods for their suppression in the brewery have been adopted, based on the assumption that conditions inhibitory to one species would be equally effective towards others. Bacteria capable of growing in wort and beer, however, vary widely in their cultural requirements and their reaction towards hop antiseptic, and it is necessary to take this into consideration when combating bacterial infection in the brewery.

In this chapter an attempt has been made to review the present position of brewing bacteriology, and an account has been given of a number of beer bacteria, together with an outline of methods available for their suppression. Some of the bacterial species recorded in earlier brewing literature have been omitted, as in some cases these have been described too inadequately to enable them to be identified to-day, whilst in others bacteria have been recorded as beer types solely on the grounds of their morphological resemblance to well-known species occurring in other habitats.

The bacteria mentioned in this chapter by no means necessarily include all the species capable of functioning as brewery organisms. It is probable that as many more still remain to be investigated; it may again be stressed, therefore, that the morphological resemblance of any bacterium encountered in the brewery to any of the species described in this paper is inadequate reason for assuming identity.

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CHAPTER XXXVIII

YEAST NUTRITION

CARBOHYDRATE AND NITROGEN REQUIREMENTS

(676) The Carbohydrates of Yeast.

The composition of wort would give a very false idea of the nutritional requirements and composition of yeast were it not realised that the cells derive the energy required for synthesis of tissues and growth from respiration or fermentation. These are both downgrade processes, but respiration is by far the better source of energy and fermentation of relatively great quantities of carbohydrates is required to replace it. Nevertheless about one-third of the dry weight of yeast consists of carbohydrates or allied substances, mainly glycogen and mannan. Yeast synthesises glycogen, possibly as an intermediate stage in the process of fermentation (Section 691), and stores the excess over current requirements as a reserve of carbohydrate. It builds up cell walls, which may constitute as much as 20% of its dry weight and consist of hemicellulose (mannan), with some glycogen in a highly polymerised state and phosphoric acid. Fat is synthesised from sugars and may, with certain yeasts and suitable growth conditions, accumulate to such an extent as to become a valuable source of this substance for food and technical purposes. The analyses in Table 235, by Ling, Nanji and Paton,[1] show that the quantities of glycogen and hemicellulose in yeast decrease during the first 30 hours of fermentation, after which they steadily increase. Production is most rapid during the period of maximum fermentation but, as the rate of the latter decreases, there is a slight fall in the percentage of glycogen, of which the granules in old yeast cells largely consist.

An ample supply of nutrients, access of oxygen, high acidity and high temperature favour the formation of fat, apparently as a reserve material. Starved cells contain none, but cells in good condition may have 10–20% on their dry matter, though the quantity is generally much less, 2–5%. Lecithin and ergosterol generally accompany the fats. Ergosterol, a secondary alcohol of ring structure, is transformed to vitamin D by ultra-violet irradiation. Lecithin is a complex ester, comprising glycerol, fatty acids, P_2O_5 and choline in its molecule.

TABLE 235.—GLYCOGEN AND HEMICELLULOSE IN YEAST DURING FERMENTATION, % ON DRY YEAST (LING, NANJI AND PATON)

Hours after pitching	Glycogen %	Hemicellulose (Mannan) %
9·0	24·34	6·60
15·5	18·34	2·53
33·0	11·20	3·80
39·5	18·00	3·95
57·0	21·87	9·24
63·5	30·35	9·38
81·0	26·02	9·25

(677) Nitrogen Requirements of Yeast.

The cytoplasm of yeast consists essentially of protein. An albumin, known as cerevesin, and a phosphoglobulin, zymocasein, have been identified, together with nucleoproteins as the chief constituent of the nucleus. The total nitrogen content of pressed yeast is usually between 1·6 and 2·0%, equivalent to between 10 and 12·5% of protein. These figures represent about 5–7% of nitrogen or 31–44% of protein when expressed as percentages of the dry matter of the yeast. A considerable quantity of nitrogen is consequently required for the synthesis of protein in newly formed yeast cells, 8 to 10 lb. of nitrogen, for example, to produce 500 lb. of pressed yeast; 100 barrels of wort with nitrogen contents of 0·10 and 0·05% would contain 36 and 18 lb. of nitrogen respectively, figures which closely correspond with the nitrogen content of worts of 1055 and 1030 specific gravity, brewed from five-sixths English malt, containing 1·5% of nitrogen, and one-sixth adjuncts. The proportion of this nitrogen available for yeast nutrition consequently becomes a question of primary importance, since any deficiency must lead to reduced reproduction and, probably, to defective fermentation.

The figures in Table 176, Section 439, show that yeast can be caused to assimilate about 60% of the total permanently soluble nitrogen of worts from English malt, but that a proportion of this is not readily utilised. The percentage assimilated in brewery fermentations is always considerably less than 60%, varying with the composition of the wort, the attenuation, the yeast and other factors. Brown[2] found that quantities ranging between 20 and 40% of the permanently soluble nitrogen were removed from worts of varying gravity in different breweries, whilst according to Nielsen,[3] 40–50% of the total nitrogen of Carlsberg Brewery worts was assimilated in most cases, the range being 34–55%. The proportions for a number of Munich worts were 30–46% for dark and 37–54% for pale worts. There should, therefore, be ample nitrogenous yeast nutrients in worts of 1050–1055 specific gravity, in the circumstances mentioned above and allowing for 40% assimilation, but there might be a shortage in worts between 1040 and 1030 for the stated yeast crop.

TABLE 236.—INFLUENCE OF NITROGENOUS NUTRIENT ON YEAST GROWTH (TAIT AND FLETCHER)

(NITROGEN AND YEAST IN MGM. PER 100 ML.)

Nitrogen				Yeast crop mgm.	Unfermented sugar %	Nitrogen % in dry yeast
Initial		Removed				
Total	Assimilable	mgm.	% of total			
1·5	0·69	0·69	46·0	250	92·6	1·1
4·4	2·02	1·93	43·8	477	88·0	1·6
9·0	4·2	4·2	46·8	750	58·1	2·2
17·8	8·2	8·11	45·6	1080	31·3	3·0
35·6	16·4	16·43	46·3	1680	5·5	3·9
41·3	19·0	18·18	44·2	1800	1·9	4·1
50·1	23·0	22·83	45·6	1977	2·1	4·6
67·7	31·2	29·55	43·7	2227	1·3	5·3
85·6	39·4	36·52	42·8	2420	1·0	6·1
127·8	58·8	49·97	39·1	2727	1·1	7·3
170·9	78·6	49·12	29·2	2550	1·1	7·8
256·5	118·0	37·85	14·2	2537	1·1	6·0
342·0	219·0	30·55	8·7	2303	1·0	5·3

The presence of an adequate supply of fermentable carbohydrate is essential for nitrogen assimilation and its exhaustion may be the limiting factor in fermentations of low gravity worts. The figures in Table 236 show the relation between yeast growth and the nitrogen content of the wort when the sugar supply is assured. They are from Tait and Fletcher's[4] experimental fermentations in solutions of invert sugar containing the necessary mineral nutrients and malt rootlet extract as a source of nitrogen and bios. They should not be taken as applying quantitatively to brewery fermentations, but illustrate the general principle that fermentations will be unsatisfactory if the supply of assimilable nitrogen is too small. The pitching rate was 88 mgm. per 100 ml. (about 0·8 lb. per barrel) and the fermentations lasted 7 days at 65° F. The apparently detrimental effect of excess of nitrogen is not so readily explained, but more recent investigations have emphasised the fact that satisfactory utilisation of any of the nutrients of wort is dependent on the presence of suitable, but very small, quantities of accessory growth-promoting substances or bios (Section 682).

L. R. Bishop has informed the writer that small scale fermentations at the Institute of Brewing Laboratories suggest that the minimum requirements of permanently soluble nitrogen for full attenuation, in presence of bios, are represented by (wort sp. gr. —1000) × 1·0 mgm. per 100 ml. of wort, but may be as much as (wort sp. gr. — 1000) × 1·8 mgm. where the fermentations are not continuously stirred. These figures indicate that nitrogen requirements are proportional to the gravity of the worts. It appears, however, that bios and, possibly, mineral requirements may be proportionally greater at lower gravities. This may explain some cases of yeast failure or progressive deterioration from fermentation to fermentation in low gravity worts.

(678) Sources of Assimilable Nitrogen.

Yeast can make use of the nitrogen of ammonia, of many ammonium salts and of some other inorganic nitrogen-containing salts. Ammonium phosphate is a good nutrient, as is ammonium sulphate if steps are taken to neutralise the liberated acidic ion. Such salts as ammonium acetate from which undissociable fatty acids would be liberated are not suitable as nutrients. Thorne[5] found that potassium nitrate functioned tolerably well, in presence of bios, in so far as growth was concerned, but that the fermentative power of the resultant yeast was unusually low. Nitrites have a poisonous effect on yeast[6] (Vol. I, Section 345). The most important nitrogenous nutrients in wort are, however, the most profoundly degraded protein derivatives, including the amino-acids and simpler polypeptides. Thorne, for example, obtained good yeast growth in synthetic media, containing adequate supplies of sugar and growth-promoting substances, with asparagine, leucine, valine, arginine, phenylalanine, glycine or tyrosine as the only source of nitrogen. These are probably among the amino-acids of wort. Moderate results were obtained with urea, uric acid, alanine, hydroxyproline, proline and histidine, but only poor results with tryptophan, cystine, certain amides and organic bases which might also occur in worts. Several dipeptides were found to be readily assimilable by yeast.

It is not yet definitely known how far up the scale of more complex protein derivatives yeast can go for its nitrogen requirements, or at what stage of complexity they cease to be able to diffuse into the cells. Though the *total assimilable* nitrogen in the wort of Table 176 was an approximately constant proportion of the permanently soluble nitrogen, there was clear evidence of reduction in the proportion of *readily assimilable* nitrogen with increasing mashing temperatures or restriction of proteolysis.

Windisch, Kolbach and Hennecke[7] found that about half the total nitrogen of wort was assimilable under favourable conditions and, of this, about half was formol titratable. This does not mean that half of the readily assimilable nitrogen is necessarily derived from amino-acids. It might be either more or less. Only half of the amino-nitrogen of such compounds as asparagine, glutamine, histidine, arginine and tryptophan is measurable by formol titration. On the other hand, a decreasing proportion of the nitrogen of more complex products of proteolysis, from polypeptides to proteins themselves, reacts to this method of analysis and would be included in the amino-nitrogen or formol nitrogen. The more complex protein derivatives, removable from the wort by adsorption on a mixture of asbestos and kaolin or by ultrafiltration, are, however, not assimilable to any measurable extent. Treatment of the wort with pepsin or papain does not increase either the assimilable or formol nitrogen. The peptone-like proteolytic products of these enzymes are apparently not assimilable. Trypsin, on the other hand, which carries proteolysis further and produces amino-acids and polypeptides, increases the assimilable nitrogen of wort The proteolytic enzymes of malt can increase both the amino- and assimilable nitrogen to a corresponding extent.

Table 237, privately communicated by Dr. L. R. Bishop, incorporates some of the most recent results of experiments with top fermentation yeasts at the Institute of Brewing Research Laboratories. It shows that the main uptake of nitrogen occurs in the first 24 hours of fermentation, and that the absorption of nitrogen by the yeast conforms with the fall in wort nitrogen.

TABLE 237.—TOTAL AND FORMOL-NITROGEN UPTAKE BY YEAST DURING FERMENTATION OF WORT OF 1055 SP. GR. (L. R. BISHOP)

(MGM. N. PER 100 ML.)

Hours fermented	Wort		Yeast		Wort		Formol N decrease % total N
	Total N	Formol N	Total N	Increase N	Total N increase	Formol N decrease	
0	133·8	45·7	4·5	—	—	—	—
15	108·8	32·6	28·0	23·5	25·0	13·3	53·2
25	71·8	10·6	70·0	65·5	62·0	35·1	56·6
62	71·5	9·4	69·4	64·9	62·3	36·3	58·2

The formol-nitrogen taken up averaged 56% of the total nitrogen absorbed, while some 5% of the latter was derived from free ammonia and about 9% was amide-nitrogen. The nitrogen accounted for in these groups amounted to 70% of the total uptake. The remainder probably consisted almost entirely of peptide nitrogen, that is of amino groups linked up to build rather more complex compounds. If these consisted of di-peptides, they would account for 30% of the amino-nitrogen, that is a quantity equivalent to the peptide-nitrogen assimilated, which would lead to the conclusion that some 26% of the nitrogen assimilated existed as free amino-acids, and about 60% as simple peptides. If the assimilated nitrogen included that of some tri- or higher peptides, the analytical figures would indicate a correspondingly greater proportion of assimilated amino-acids and a smaller proportion of peptides.

The available experimental evidence shows that yeast preferentially and most readily assimilates the simplest protein degradation products, the amino-acids, dipeptides and, possibly, some tripeptides and other rather more complex compounds with greater difficulty, but cannot utilise the nitrogen of peptones or of still more complex products of protein breakdown. The conditions of fermentation, however, have considerable influence on nitrogen assimilation. This may result either from its extension to more complex substances or from more complete utilisation of the simple compounds. Brown's differentiation of readily assimilable nitrogen from that which is utilised with difficulty might be interpreted either way. Taxner's[8] experiments showed that the reproduction of yeast could be increased up to about 20-fold in synthetic media containing asparagine as the only source of nitrogen, if the latter were added in small quantities as fermentation proceeded, as in yeast manufacture, all other conditions being as favourable as possible.

(679) Mechanism of Amino-acid Assimilation.

Ehrlich[9] found that fermentations of nutrient solutions containing α-amino-acids as the only source of nitrogen yielded the corresponding alcohol. Thus inactive *iso*-amyl alcohol was obtained from leucine, according to the equation :

$$(CH_3):CH{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH + H_2O = (CH_3)_2:CH{\cdot}CH_2{\cdot}CH_2(OH) + CO_2 + NH_3$$

He concluded that the amino-acids were not assimilated by the yeast as such, but only the ammonia split off was utilised in the synthesis of yeast proteins. Ehrlich's fermentations were carried out under rather artificial conditions, but Thorne,[10] by adopting experimental conditions that approximated as closely as possible to brewery fermentations, showed that this manner of regarding the utilisation of amino-acids was probably correct in its application to practice. He confirmed the production of higher alcohols from tyrosine, tryptophan, phenylalanine, valine and of succinic acid from glutamic acid and of γ-butylene glycol and succinic acid from arginine. These reactions require the presence of living yeast and are not brought about by zymase preparations. Ehrlich[11] came to the conclusion that the succinic acid found in fermentations was derived from glutamic acid, formed with other amino-acids from dead yeast cells and utilised by others. The transformation may be expressed in the simplest manner as :

$$\mathrm{COOH{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH + O_2 =}$$
$$\mathrm{COOH{\cdot}CH_2{\cdot}CH_2{\cdot}COOH + CO_2 + NH_3}$$

The reactions are, however, more complicated, comprising oxidation-reduction processes (Neubauer and Fromherz[12]), involving the production of a ketonic acid, followed by its decarboxylation to the corresponding aldehyde, which would then be oxidised or reduced to an acid or alcohol, respectively.

TABLE 238.—INHIBITION CONSTANTS OF PRODUCTS OF DEAMINATION OF AMINO-ACIDS (R. S. W. THORNE)

Inhibitor	Amino-acids from which inhibitor is derived	Inhibition Constants	
		Growth	Fermentation
Methyl alcohol	—	0·49	0·47
Ethyl alcohol	—	1·00	1·00
n-propyl alcohol	—	20·1	14·3
iso-propyl alcohol	—	1·49	1·39
n-butyl alcohol	—	7·60	3·65
iso-butyl alcohol	valine	7·32	4·63
n-amyl alcohol	*isol*-eucine	33·7	26·1
iso-amyl alcohol	leucine	29·0	24·0
β-phenylethyl alcohol	phenylalanine	41·7	27·7
tyrosol	tyrosine	5·4	5·5
tryptophol	tryptophan	112	62·7
ethylene glycol	—	0·43	0·30
glycerol	—	0·61	0·17
succinic acid	glutamic acid	nil	nil

It seems necessary to explain the varying efficiency of amino-acids as yeast nutrients either by (1) differences in the rate at which yeast is able to deaminate them or (2) varying inhibitory action of the residues, mostly higher alcohols, left after utilisation of the ammonia produced. Thorne[13] found that the residual products varied greatly in their inhibitory action. This is very low for the normal by-products, glycerol and succinic acid. The low value for tyrosol is also noteworthy, because tyrosine is known to constitute an appreciable proportion of the assimilable nitrogen of wort. At the other extreme, tryptophol, which would be produced from tryptophan, has an inhibitory value 60 to 110 times greater than ethyl alcohol. The inhibition constants for growth and fermentation found are given in Table 238, from which it will be noted that the effects on growth are greater than those on fermentation.

(680) Nitrogen Assimilation in Wort.

Nitrogen assimilated from wort by yeast may be classed as " potentially assimilable " when it is derived from simple peptides which are broken down to amino-acids in the cells, then deaminated and the ammonia assimilated, or as " immediately or readily assimilable," when it exists in the form of ammonia, amino-acids or simple amides, which have merely to be deaminated. The fact that only half of the nitrogen readily assimilated by yeast is formol titratable suggests that the quantity of the second class is comparatively low in wort. Hopkins[14] pointed out that the conditions of nitrogen assimilation in wort resemble those in the manufacture of yeast, in that there would seem to be a continuous presentation of small quantities of nitrogen. While the immediately assimilable amino-acids are being deaminated, the ammonia present is being utilised and replaced by that from the amino-acids. By the time simple amino-acids and amides have been utilised in this way, it may be assumed that diffusible di- and tri-peptides have been broken down by appropriate enzymes, thus supplying a further quantity of amino-acids. A mechanism of this kind would prevent accumulation of an over-dose of immediately assimilable nitrogen, from which would be produced an excessive quantity of deleterious by-products within the cell, allowing insufficient time for their excretion. The general experience that yeast reproduction is less satisfactory in strong worts of 1070 specific gravity and above than in others between 1045 and 1060 may also be explained in other ways. It is probable that its normal activities are checked by physical factors operating in the stronger worts. Among these may be the osmotic pressure set up by the greater quantity of dissolved sugars, which would tend to draw water from the cells. Many other factors may, however, influence assimilation and it is difficult to ascribe to them their relative significance.

The p_H value of the medium is not a factor of major importance in nitrogen assimilation and yeast growth, within the range met with in brewery fermentations. The optimum has been placed at between p_H 3·8 and 4·0. Taxner,[8] using asparagine as source of nitrogen, found that the initial p_H of the medium was more important than that attained later and placed the optimum between p_H 3·57 and 3·87. The temperature of fermentation, within the normal range, also appears to have little influence on the limit of assimilation, though it determines the time required to reach that limit, after which there may be a slight increase in wort nitrogen due to yeast autolysis (Section 685). This occurs more rapidly at higher temperatures, an increase in the nitrogen content of wort having been found, for example, after 9–10 days' fermentation with a bottom yeast at 54° F. and after 4–5 days when the fermentation was carried out at 77° F.

MINERAL NUTRIENTS AND GROWTH FACTORS

(681) Mineral Requirements of Yeast.

An idea of the mineral requirements of yeast can be gained by analysis of its ash and it is on this basis that the quantities of salts for synthetic nutrient media have been calculated. The quantity of ash varies greatly, usually between 7 and 10% of the total dry matter or between 2 and 3% of pressed yeast. The variations in the proportions of its several constituents are so great that it would be difficult to give an average analysis. Two analyses by Euler and Lindner[15] are given in Table 239, together with the percentages calculated on 100 grams of the Munich yeast in a pressed condition, assuming 3% of ash. The mineral matter extracted from malt by distilled water corresponds very closely in the proportions of the various ions with those of yeast ash, but here again it would be difficult to give an average analysis. Analyses of the ashes of two all-malt worts of 1048 specific gravity, one made with distilled water and the other with a very hard liquor, have been derived from the data in Table 130, Section 385, for purposes of comparison. These give the constituents of 2 litres of the worts, the liquor used for the second being B3 of Table 130.

It will be noticed how closely the mineral constituents of 2 litres of the distilled water wort correspond with those of 100 grams of pressed yeast. 100 grams of yeast per 2 litres corresponds

TABLE 239.—ASH OF YEASTS AND MINERAL CONSTITUENTS OF WORTS

	Yeasts			Worts 1048 sp gr.	
	% of ash		g. in 100 g.	grams in 2 litres	
	Munich	Weihenstephan	Munich pressed	Distilled water	Hard water
Potassium, K_2O ..	38·45	26·07	1·15	1·20	1·20
Sodium, Na_2O ..	—	2·26	—	—	—
Calcium, CaO ..	2·85	7·58	0·09	0·09	0·03
Magnesium, MgO ..	5·8	6·34	0·17	0·43	0·40
Iron, Fe_2O_3	0·51	0·70	0·01	trace	trace
Phosphate, P_2O_5 ..	48·19	54·31	1·45	2·03	1·59
Sulphate, SO_3 ..	0·62	0·31	0·02	0·30	1·01
Silica, SiO_2	1·26	0·92	0·04	0·3	0·1

with 18 lb. per barrel, two or three times the normal reproduction in brewery fermentations. The wort would consequently appear to be an ideal nutrient medium for yeast, if it could be judged on the analysis of its ash. This is, however, not the case, particularly in respect of its phosphate content. A considerable proportion of the P_2O_5 exists in organic combination in wort and is, presumably, not assimilable by yeast. According to analyses by Hopkins and Amphlett[16] rather more than half of the phosphate of a particular wort mashed with distilled water was inorganic and probably assimilable. When the same malt was mashed with a very hard water containing 78·6 parts per 100,000 of calcium sulphate and 11·7 parts per 100,000 of calcium carbonate about two-thirds of the phosphate was present in inorganic form in the wort, though the total quantity of P_2O_5 was much less owing to precipitation with calcium, and the inorganic phosphate was reduced, as shown in Table 240.

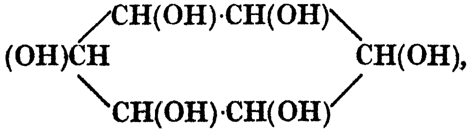
TABLE 240.—PHOSPHATES IN WORT, 30% MALT MASH
(HOPKINS AND AMPHLETT)

(MGMS. PER 100 ML.)

	Distilled water	Hard water 28 mgm. Ca per 100 ml.
Phosphate as P_2O_5. Inorganic	88·7	81·7
„ „ Organic	71·7	44·7
„ „ Total	160·4	126·4
Total calcium, as Ca	6·3	15·4
p_H	5·45	5·32

It may be assumed from these analyses that at least 1·0 gram of inorganic P_2O_5 would be present in 2 litres of both the worts of Table 240. This is equivalent to that in the ash of yeast produced at the rate of 12 lb. per barrel. The P_2O_5 content of the 1048 specific gravity wort should be ample for normal yeast production. The figures obtained in this approximate manner are in agreement with experience, according to which about 0·05% of total P_2O_5 in wort is usually adequate for healthy fermentation. An all-malt wort of 1055 specific gravity may contain between 0·07 and 1·11% of P_2O_5, but this would be materially reduced by the use of flakes, grits or sugar, since maize and rice products only contain about 0·1–0·2% of P_2O_5 and sugars much less, compared with 1% in malt. " Yeast Foods " containing soluble inorganic phosphates may consequently be useful if shortage through reduction of gravity or use of adjuncts occurs. Experiments have shown that yeast increase is proportional to the phosphate content of the medium, when this is low and adequate quantities of all other nutrients are present. The yeast crop does not, however, increase proportionally with the phosphate when this is present in excess. The analyses of fully fermented strong beers in Table 241 show that the phosphate supply was ample. This is usually the case in moderate gravity worts and the same applies to the potassium, magnesium and calcium requirements of yeast, but should any shortage of these occur, it is a simple matter to add salts to the liquor and so prevent the yeast degeneration or poor fermentations that must follow shortage of any of the nutrient requirements of yeast. The presence of minute quantities of magnesium and manganese ions is also held to be necessary in fermentation.

The organic phosphorus compounds in barley and malt are of great physiological interest, in that they supply on hydrolysis during malting, not only a considerable proportion of the inorganic phosphate requirements of yeast, but also constituents of bios and certain enzyme systems (Sections 689 and 692). Thus the hydrolysis of phytin, which is the main phosphorus reserve of plants and generally regarded as the calcium and magnesium salt of the hexaphosphoric ester of inositol, yields inositol or Bios I,



$$\mathrm{(OH)CH}\begin{matrix}\diagup\mathrm{CH(OH)\cdot CH(OH)}\diagdown\\ \diagdown\mathrm{CH(OH)\cdot CH(OH)}\diagup\end{matrix}\mathrm{CH(OH)},$$

while nucleic acid contains adenine, a constituent of cozymase, in its complex as well as other nitrogen groups linked with four molecules of the pentose sugar, ribose and four molecules of phosphoric acid. Phosphoric acid is also split off from lecithin, which exists as a phosphorus- and nitrogen-containing fatty acid ester of glycerol.

TABLE 241.—ANALYSES OF ALL-MALT STRONG BEERS

(ASH, ETC., AS GRAM PER 100 ML.)

	Burton Strong Ale 30 years in bottle	Dublin Export Stout 10 years in bottle
Original gravity	1108·36	1070·49
Present gravity	1024·15	1011·15
Alcohol gr. per 100 ml.	8·9	6·1
Organic solids	9·1	5·0
Apparent maltose	2·7	—
Nitrogen × 6·25	1·15	0·79
Acid as lactic	0·34	0·45
Volatile acid	0·04	—
*p*H	3·78	—
Ash	0·46	0·34
Total phosphates, P_2O_5	0·14	0·10
Inorganic phosphate, P_2O_5	0·12	—
Sulphates, SO_3	0·11	0·03
Chlorine	0·04	—
Magnesium, MgO	0·05	0·03
Calcium, CaO	0·05	0·01

The phosphates are largely used by the yeast in the synthesis of organic complexes, which include inositol, adenine and probably other constituents of the organic phosphorus compounds of malt in their make-up. Macfarlane[17] found that of the 0·6% of phosphorus in a certain pressed yeast, about 50% could be extracted with trichloracetic acid, approximately 15% as orthophosphate, 25% as a labile phosphoric acid, containing inorganic pyrophosphate, and 10% in organic combination, probably including Warburg's yellow enzyme (1%) and adenosine triphosphate (2%). Very little, if any, of the phosphorus exists as hexosephosphate (Section 688). Approximately 30% of the phosphorus of yeast is present as a complex containing iron, nucleic acid and metaphosphoric acid. It may be supposed that this is enzymically dephosphorylated in fermentation, with the production of orthophosphate and nucleic acid. Lecithin is also found in yeast and, among organic compounds of special significance, though present in very small amount, are the yellow enzyme of respiration and cocarboxylase, in which phosphate is combined with vitamins B_2 and B_1 respectively (Sections 692 and 689) and cozymase.

(682) Growth-promoting Substances.

This term is applied to substances which themselves are generally held to have no nutritive value, but must be added in very small quantity to otherwise complete and suitable nutrient media in order to effect the normal growth of an organism. It does not appear possible strictly to draw the line between substances that may be required in minute quantity in the synthesis of such a complex substance as protoplasm, thus being true nutrients, and others that may act in a merely regulatory manner. For example, Nielsen and Hartelius[18] came to the conclusion that four of the 34 amino-acids which they studied should be regarded as growth-promoting substances, because, when present in extremely low concentration in synthetic nutrient media, they increased the multiplication of yeast to an extent quite out of proportion with their quantity. The amino-acids in question were β-alanine, lysine, arginine and glutamic acid. Of these, β-alanine, $NH_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$, was the most active, 1 mgm. in 10 litres producing maximum effect, while 1 mgm. in 200 litres still had a distinct stimulating effect. They found, however, that it acted as a growth-promoting substance only in the presence of asparagine or aspartic acid and at higher concentrations it might even have a depressing influence on reproduction. Williams and Rohrman had previously demonstrated that β-alanine actively stimulated the growth of some yeasts, though it cannot be utilised as a source of nitrogen.

Wildiers[19] discovered in 1901 that satisfactory yeast growth could not take place in the absence of some " growth-promoting " substance that existed in wort and commercial peptones and was extractable from yeast, was soluble in water but not in alcohol. To this he gave the name Bios. There is thus an analogy between the nutritional requirements of micro-organisms and animals, and probably actual identity in certain of the constituents of bios and vitamins. Bios-containing extracts have been fractionated, one fraction, Bios I, differing from the other, Bios II, in that it gave a barium salt which was insoluble in alcohol (Lucas[20]). Bios II was then divided in two fractions by differential adsorption on fuller's earth or active carbon. Bios was thus shown to consist of at least three substances, which Lash Miller[21] called Bios I, Bios IIA and IIB. Eastcott[22] identified Bios I as *meso*-inositol. The fraction that is not adsorbed on fuller's earth was called Pantothenic acid by Williams,[23] while Kögl and Tönnis[24] claim to have isolated the pure Bios IIB in a crystalline form from egg yolk. Its activity was so great as to be detectable at a concentration of 1 mgm. in 400,000 litres of a synthetic nutrient medium. Addition of 0·00167 mgm. to a suspension of yeast in a glucose mineral medium increased the rate of growth 7 times. Its estimated concentration in fresh yeast was 1 in 36,000,000.

Bios is very widespread in plant and animal products, but the extremely small quantity in which it exists in any of them has made it difficult to elucidate the nature of its components, while the adoption of varying criteria of activity and use of different yeasts for test purposes have introduced apparent contradictions in experimental results. The so-called "Z-factor," separated from yeast by Euler,[25] was regarded as an accelerator of fermentation. Its relation with bios is not clear, though growth and fermentative activity are closely related. Bishop and Rainbow[26] adopted Miller's nomenclature for three fractions which they isolated after 200,000 times concentration of bios extracted from air-dried egg yolks. They agreed that Bios I was *meso*-inositol, and found that Bios IIA and IIB were comparatively simple nitrogenous organic compounds, but they have come to the conclusion that their Bios IIA was not identical with that of Miller. It appeared to be the same as Williams' "panthothenic acid," and to consist of β-alanine linked with an unknown hydroxy-acid, while Miller's IIA was β-alanine. Bios IIB was apparently similar to Kögl's "biotin." Other authors have found that some yeasts require aneurin (Vitamin B_1), or Vitamin B_6, in addition to one or other of the bios fractions, which are adequate, in suitable proportions, for maximum growth of most races.

It has been found that yeasts vary in their bios requirements and this, according to Copping,[27] is related to their ability to respire oxygen, the most feebly respiring yeasts having the greatest need. Some yeasts can grow in synthetic media free from bios and appear able to synthesise their requirements. These, generally wild yeasts, respond poorly to added bios, while those which will not grow satisfactorily in its absence react markedly. Thorne noticed that the pure culture top fermentation yeast used in his experiments stood in greater need of bios than his culture of Frohberg yeast. Nielsen[28] found that the pure Carlsberg yeast, with which he experimented, could not grow in pure artificial solutions and concluded that it was unable to synthesise bios. Neither Bios I nor Bios IIA affects the growth of yeast alone, but both enhance the growth-promoting action of Bios IIB, but it appears that different yeasts have varying requirements in respect of the three fractions and may be supposed to synthesise those which it is not necessary to add to the nutrient medium. All the yeasts examined by Rainbow[35] appeared to require inositol and Bios IIB to multiply to an extent approaching maximum growth in malt wort. Some required, in addition, the IIA fraction, while others were satisfied with synthetic β-alanine in its place. Others, again, grew well without either IIA or β-alanine. The nature of the medium also influenced the bios requirements of yeast in many cases. Aspartic acid, in particular, when used in place of ammonium phosphate, increased the effects of IIA and IIB or β-alanine with some yeasts, but not with all.

Nielsen[3] found that the component that influenced the production of yeast dry substance existed in appreciable quantity in barley and increased during malting, but not by more than 50%. Thus, in an experiment at various stages of malting, 2·2 mgm. of dry yeast substance were obtained in nutrient media without growth-promoting factors, while 7·0 mgm. were produced with a small addition of barley extract, 9·6 mgm. with malt extract after 9 days' growth and 8 5 mgm. with an extract of finished malt, from which rootlets had been removed. The latter provide a a valuable source of bios, which is obtained in adequate concentration for experimental purposes by adding 10 or 20% of malt rootlet extract of approximately 1030 specific gravity to a sugar and mineral nutrient solution in which yeast growth is otherwise very imperfect. Very little change was found to occur in the activity of growth-promoting substances from mash tun wort to refrigerator wort, addition of hops caused no increase, while rice and maize contained very little. Bishop and Rainbow,[26] however, found that a little bios was contributed by hops and that there was no difference in the bios content of malts after 5, 7 or 9 days on the floor.

Bishop and Rainbow pointed out that about 15 mgm. per litre (1 grain per gallon) of inositol was required to give good yeast growth in sugar and salt solutions, whereas only 0·2 mgm. per litre (15 thousandths of a grain per gallon) of their most active preparations of Bios IIA and IIB was necessary. Yeast does not increase in synthetic media by more than 50% in their absence, but a 70-fold multiplication was obtained when the above quantities were added. The bios content of malts varied and there was an apparent shortage in worts of 1030 specific gravity from some malts, but excess was present in higher gravity worts, above 1050. One particular malt, Dixon's Enzymic malt, was found to have considerably greater bios activity than ordinary malts, nevertheless yeast growth was limited in worts made from it on account of their low fermentability. This malt is, however, only used in small proportion with other malts in practice, when its bios content has full play. This indicates the necessity of making sure that all other nutrients are present in adequate quantity in tests for bios based on yeast increase.

The bios shortage detected in some malts, particularly in those that had been stored a few years, appeared to be in Bios IIA. Similar signs of a shortage of this fraction were found in some beers, from which it was argued that shortage of IIA, though not apparent in the primary fermentation, might show up in the after-fermentation in which yeast growth continues towards the greater amount found in the laboratory swirling tests. Results obtained by these authors are given in Table 242 to illustrate the method used to detect bios in beer. The figures in column 2 are yeast growths as percentages on the nephelometer after 48 hours' growth in 50 ml. of synthetic medium to which 5 ml. of dealcoholised beer had been added. Those in the other columns represent the growths after addition of a quarter wort dose of concentrates of Bios IIB or IIA. Signs of IIA shortage appear in beers 4 and 5.

TABLE 242.—BIOS FRACTION TESTS ON COMMERCIAL BEERS (BISHOP AND RAINBOW)

Beer	Beer alone	Beer + ¼ dose IIB	Beer + ¼ dose IIA
1	75	83	90
2	62	76	87
3	102	124	149
4	54	74	138
5	41	55	108
6	56	69	94

The serious effects of lack of nitrogenous nutrients on the health of yeast is generally recognised, but the outcome of these investigations appears likely to be a demonstration of the even greater significance of bios deficiency, which may occur at the same time or independently. To it may, for example, be attributed many cases of wild yeast and bacterial development, which is held in check or prevented by vigorous primary yeast and healthy fermentation. Progressive change in the appearance or behaviour of pitching yeast may also be due to gradual replacement of one race by another which has smaller bios requirements. A suitable balance between bios and the ordinary nutrients may be required for adequate utilisation of the latter.

(683) Vitamins of Yeast.

Yeast is among the richest sources of vitamins, but it is not known how many of these essential factors in diet are present, probably six at least, of which B_1 and B_2 are the best known. B_3, B_4 and B_6 have also been detected. They are intimately associated with the enzymic systems of yeast; B_1, identified as aneurin, as a constituent of cocarboxylase, B_2, or lactoflavin, in Warburg's yellow enzyme, while B_3, nicotinic acid amide, exists in cozymase (Sections 689, 692). The human requirement of B_1 and B_2 is about 1 mgm. per day and this would be obtained in about 10 grams of brewery yeast. The fat-soluble vitamin D, occurring in cod liver oil, is not itself present in yeast but can be obtained by irradiation of ergosterol with ultra-violet light and hence is present in irradiated yeast, which contains ergosterol in conjunction with its fat.

The chief sources of vitamins of the B series are living plants. They occur in barley, malt and many cereal products, particularly in the aleurone layer and, being water-soluble and fairly stable to heat, exist in wort. Yeast grown in malt wort is rich in these vitamins, but bakers' pressed yeast, produced in molasses media, contains very little. It would appear from this that yeast draws its vitamins from the culture medium and it is uncertain whether brewery yeast can synthesise them. Drummond and Whitmarsh,[29] however, obtained evidence of the synthesis of vitamins B_1 and B_2 by Logos yeast, grown in vitamin-free media. This yeast grows well in a sugar-salt medium, free from bios. The results with a strain of *S. cerevisiae*, which required a small addition of bios in the medium, showed that little, if any, vitamin B_1 was produced and only small amounts of B_2. The B_1, added with the malt extract used as source of bios, was removed from the culture medium by the yeast, but the amount of B_2 remained about the same. Some yeasts require additions of B_6, others do not.

Yeast can be dried *in vacuo* at 100°–130° F. without significant depreciation in vitamin potency. It has been found that vitamin B_1 was stable to dry heat at 248° F. for 24 hours, but was destroyed after 144 hours in a dry, or after 24 hours in a moist, medium at 212°. Both B_2 and B_4 were destroyed by dry heat at 248° F. in 25 hours (Keenan and Kline[30]). Rapid advances have been made in the chemistry of the vitamins during the last few years, several of them having been obtained in a pure or approximately pure state, while synthetic products of comparable properties are made for therapeutic use. Thus vitamin B_1 was isolated in a crystalline form from rice polishings by Jansen and Donath (1927) and its equivalent is produced commercially. B_2 is also prepared synthetically as lactoflavin. The formulae of both these, in phosphate combinations, are given in Sections 689 and 692 as constituents of cocarboxylase and Warburg's yellow oxidation enzyme, respectively.

BY-PRODUCTS OF FERMENTATION AND BEER FLAVOUR

(684) Excretory Products of Yeast Metabolism.

Yeast nutrition necessarily involves excretion of breakdown products of sufficiently small molecular size readily to diffuse through the cell walls. These include the alcohol and carbon dioxide of normal fermentation, together with the small quantity of by-products that accompany them and larger amounts of the same and other substances derived through degradation of structural elements of the cells under pathological or morbid conditions. Substances excreted during the early stages of fermentation consist almost entirely of alcohol and CO_2, which Bishop[31] showed diffused through the cell walls in liquid state and was changed to bubbles of gas outside the cells. In these early stages, the diffusion of nitrogenous and other substances is very slight, but, after 52 hours in experimental fermentations, Tait and Fletcher[32] found a diminution of the weight of yeast, though the number of cells remained approximately the same. Lampitt[33] found that the excreted nitrogen consisted of high molecular protein derivatives (peptones) and that 33% of the nitrogen of yeast might be excreted in some cases, before the yeast died.

Isolation of various intermediate products by modification of the normal conditions of fermentation (Section 687), suggests that these may also be found in traces in brewery fermentations and in larger quantity in the event of any abnormality. The quantity of glycerol ordinarily produced approaches 3% of the sugar fermented, though it may be raised to 20% in the presence of sufficient sulphite or when the fermenting liquid is made alkaline. The maximum yield of succinic acid in alcoholic fermentation amounts to between 0·2 and 0·6% of the sugar utilised. Such a drastic alteration in conditions as is required to produce these in any quantity cannot occur in wort and normally the amounts of accessory products from amino-acids are so small as almost to defy detection by analysis. Some of the higher alcohols and esters that might be formed in beer, particularly after long storage, have such strong flavours and aromas that they must have an influence on the character of beer, even though they only occur in minute quantities.

Ehrlich, for example, detected tyrosol and tryptophol in beer and both of these have bitter flavours, tyrosol being intensely bitter. They may be supposed to be produced respectively from tyrosine, $OH{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$, and tryptophan,

$C_8H_6N{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$. Lüers and Leiss[34] had an opportunity to determine the nature of the by-products in beer by analysis of the water used to wash the CO_2 from a long series of brews in a Nathan brewery and in which comparatively large quantities of the volatile products of the primary fermentation had accumulated. They established the presence of amyl alcohol and hydrogen sulphide in largest quantity, the latter having probably been formed from cysteine, a sulphur-containing amino-acid present in wort. *Iso*-butyl, *n*-propyl and *iso*-propyl alcohol were found in diminishing quantities. Acetic acid was the most prominent volatile acid detected, while propionic and butyric acids, together with the amyl ester of caproic acid, were also present. The esters of these acids are well known for their ethereal odours. The most abundant were ethyl and amyl esters. Acetaldehyde and furfuraldehyde were also detected. Such an array of by-products is very remarkable and it is not surprising that the aroma of beer should vary so widely, with such possibilities of variation in their proportions.

One of the most interesting by-products so far isolated is diacetyl, $CH_3{\cdot}CO{\cdot}CO{\cdot}CH_3$, which Shimwell and Kirkpatrick found to contribute to the so-called "Sarcina odour" in beer (Section 670). It is produced by *Streptococci*, probably as a secondary product by oxidation of acetyl-methyl carbinol. Pure diacetyl has a pungent odour, quite different from the "butter aroma" which it gives when diluted several hundred thousand times. Claussen and Trolle have found that if one part in 2½ millions is added to lager beer, the latter takes on a character virtually indistinguishable from that of "sarcina sickness." This example emphasises the minute quantities of by-products required to give characteristic flavours or aromas, and the great variations produced by dilution. The esters behave in a similar way on dilution, the pleasant aroma produced being quite different from that of the very pungent pure product. The character of beer depends in no small degree on traces in varying proportions of such substances produced by the different yeasts and bacteria present.

Though most of the aroma-giving substances identified contain no nitrogen, the proteins of barley and amino-acids derived from them are believed to play an essential part in their formation during fermentation. Consequently the flavour and aroma of beer must depend to some extent on the nitrogen content of the barley and the manner in which its proteins have been broken down during malting and mashing.

The excretory products of yeast metabolism act in varying degree as inhibitors of growth. In the case of alcohol, this can be detected at as low a concentration as 0·7% (Tait and Fletcher), but yeast growth is not stopped until the concentration reaches about 8·5%. Carbon dioxide is not of such importance as experiments with pure CO_2 have suggested and has probably little if any practical significance as an inhibitor of yeast growth. Whatever effect the excretion of acids might have is more or less nullified by the buffer action of wort constituents. These prevent the p_H of the wort from falling much below 4·0, unless bacterial acid production supervenes. The optimum value for yeast growth, 3·8–3·9, is rarely reached while the yeast is active. The contents of the yeast cells themselves are so highly buffered that their normal p_H of 5·9–6·0 appears to be influenced very little by normal changes in external conditions.

(685) Autofermentation and Autolysis.

Some of the effects of autofermentation and autolysis were described in Sections 589 and 590. The breakdown of glycogen in the yeast cells when the normal course of metabolism is upset by lack of nutrients, generally referred to as autofermentation, apparently follows a course very similar to that of fermentation. It ultimately leads to depletion of the reserve materials of the cells and death supervenes. The organised structure of the cells, in which enzymes and substrates are held to be spatially separated, then disappears and complete breakdown follows. This process of autolysis, so-called because the cells appear to dissolve themselves, is also set in motion by heating yeast above about 77° F. Proteases, amylases and other enzymes digest the cytoplasm and other cell constituents and this is accompanied by a large increase in the amount of matter extractable from the yeast by water and a nearly parallel increase in the quantity of protein degradation products, a fact of which advantage is taken in the manufacture of yeast extracts, with the aid of salts that activate the breakdown. Activity of the zymase group of enzymes, which are desmo-enzymes (Section 121), is rapidly destroyed, since their existence appears to depend on that of the cytoplasm to which they are bound.

(686) Summary.

All the nutritional requirements of yeast are adequately supplied by a malt wort of moderate gravity, provided the enzymic processes have been carried far enough during malting and that those which normally occur during mashing are not unduly restricted by high temperatures. These requirements include a sufficient supply of fermentable sugar, with assimilable nitrogen, salts, particularly phosphates, metallic ions of potassium, sodium, calcium, magnesium and iron, part of which may be derived from the liquor, though the greater part is made available through enzymic breakdown of organic phosphates during malting and mashing. It appears that these processes also provide the necessary growth-promoting substances, bios, and vitamins required by the yeast for synthesis of its own enzymic mechanism. These requirements appear to demand a reasonably full degree of modification in the malt, or a mashing process designed to facilitate further breakdown when modification is restricted. The drawbacks of over-modification seem to result mainly from reduction in the colloidal properties of malt constituents, leading to lack of fulness and head-retention in the beer, rather than from excessive production of nutrients, which was formerly regarded as responsible for development of wild yeasts and bacteria.

Fermentation of wort cannot be carried out satisfactorily in the absence of sufficient nitrogenous and mineral nutrients to provide for yeast reproduction. If these are lacking, some may be supplied in the form of yeast foods which generally consist of fully degraded protein derivatives, obtainable from malt rootlets, and phosphates. An adequate bios supply is essential for yeast growth, and the presence of vitamins B_1 and B_6 is necessary for some yeasts. B_1 and B_2 take part with phosphates in the elaboration of coenzymes and the enzymic systems concerned with respiration. The vitamins are believed to be entirely absorbed from wort during the course of fermentation and are absent in fully attenuated beer from which all yeast is removed. The question of their existence in less fully attenuated or stronger beers does not appear to have been investigated. Processes by which vitamin-containing yeast extracts are added to the fermented beer are used to some extent in the production of "vitamin beers."

Excretory products, which are a necessary concomitant of the nutrition of yeast and its ordinary metabolic processes, have a most important influence on the flavour and aroma of beer. Many of those which contribute to bouquet or, in some cases, to bitter flavours are produced in the chemical reactions accompanying the abstraction of ammonia from amino-acids. The ammonia is the essential nutrient substance utilised by the yeast in synthesis of proteins, the higher alcohols are the residues not required and therefore excreted into the medium, where they may act as inhibitors of growth. Bacteria and wild yeasts produce substances of pleasant or objectionable aroma and flavour, which are quite distinct from those formed in normal fermentation or acidification. Among these are some which contribute to the character of old stock ales, produced by wild yeasts or *Brettanomyces*, and "sarcina odour," due to diacetyl.

Apart from their role as buffers or regulators of the reaction of the mash and as yeast nutrients, the salts of wort play an important part in contributing to the flavour of beer and its use as a thirst quencher, for which the chlorides derived from the liquor and in small quantity from the malt are particularly valuable, as they replace the salt lost in perspiration. The salt regulates the composition of the blood and, according to J. S. Haldane, prevents the cramps and headaches which so frequently result from its deficiency after violent muscular exertion. Phosphates are as essential to man as they are to yeast. Phosphorus is necessary for the repair and upkeep of bones, central nervous system and all cell nuclei. That which is left by the yeast in beer after all its needs are supplied in fermentation is a useful source of this element in human nutrition. A pint of moderately strong beer, 1048, will ordinarily supply about a sixth of the average man's daily requirements.

It must be noted that yeast growth may be limited and the liability to development of wild yeasts and bacteria increased by deficiency of the ordinary nutrients, particularly nitrogen, of bios or of both. Phosphates are less likely to be deficient. Sugar may become the limiting factor in presence of excess of assimilable nitrogen and bios. It appears that a considerable proportion of the available bios and nitrogen is absorbed by yeast in the earlier stages of its growth for utilisation as fermentation proceeds. Growth may continue for some time after the nutrients of the medium are exhausted, the nitrogen content of the yeast falling at the same time. Yeast activity is closely related with its nitrogen content, being greatest when this is from 7 to 8% on dry matter and practically nil if the nitrogen content falls to 2·5%.

Special consideration should be given to the nutrient requirements of yeast when making up the materials for a brew. If the suggestion (Section 677) that the minimum requirements for full growth and attenuation are about (wort sp. gr. — 1000) × 1·8 mgm. of nitrogen per 100 ml. of wort should prove to hold for brewery fermentations, it would mean that these could not be supplied unless the malt contained 1·35% of nitrogen, cf which about 40% represented the permanently soluble nitrogen of all-malt wort. It is consequently desirable to select malts with proportionally higher nitrogen content when adjuncts which yield no wort nitrogen are used.

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CHAPTER XXXIX

THE NATURE OF FERMENTATION AND RESPIRATION

FERMENTATION

(687) Cell-free Fermentation.

Fermentation is one of the methods by which yeast gains the energy required for its life processes. The amount obtainable by splitting sugar in fragments is not nearly so great as that available through respiration, but it is sufficient to enable the cells to multiply slowly in complete absence of oxygen. The enquiry as to how the sugar is broken down has provided one of the most fascinating and difficult problems of biochemistry. Gay-Lussac's equation

$$C_6H_{12}O_6 = 2CH_3{\cdot}OH + CH_2{\cdot}2CO_2$$

represents nothing but the raw material and stable end-products of fermentation. Pasteur (1857–1858) proved that it did not tell the whole story and that succinic acid and glycerol normally accompanied the alcohol and carbon dioxide. It became clear that fermentation must involve intermediate or side reactions, but it was not until 1897, when Edouard Buchner[1] found that sugar could be converted to alcohol and CO_2 by a liquid pressed from yeast and entirely devoid of cells, that experimental proof was forthcoming of the already suspected part played by enzymes in fermentation. Henceforth it was possible to regard it as the result of the activity of biochemical catalysts and to study its chemistry and mechanism apart from the complications involved in the life of the cell.

The intermediate reactions follow each other so swiftly and completely in normal fermentations that it is only by upsetting the gearing between them that their products can be caused to accumulate to such an extent as to reveal stages in the breakdown of sugar, but it has been found possible to do this in fermentations with living yeast by methods which have provided commercial processes for the manufacture of glycerol, aldehyde and other substances. Thus, aldehyde can be trapped as the stable aldehyde sulphite by addition of bisulphites to fermenting sugar. Glycerol becomes, at the same time, a major product instead of an insignificant by-product.

$$C_6H_{12}O_6 = CH_2OH{\cdot}CHOH{\cdot}CH_2OH + CH_3{\cdot}CHO + CO_2$$

The normal course of fermentation can also be upset by fermenting in alkaline solutions, when glycerol is obtained in high yields with acetic acid, alcohol and CO_2. Even such a small change in reaction as is caused by adding calcium carbonate, which brings the p_H of the liquid to 6·7, is sufficient to cause the separation of pyruvic acid, $CH_3{\cdot}CO{\cdot}COOH$, in the form of its calcium salt. Fermentation in alkaline solution is represented by

$$2C_6H_{12}O_6 = 2CH_2OH{\cdot}CHOH{\cdot}CH_2OH + CH_3{\cdot}CH_2OH + CH_3{\cdot}COOH + 2CO_2$$

These reactions and many others were discovered in cell-free fermentations and afterwards applied to fermentations with living yeast. The " yeast juice " or " press juice " of Buchner is obtained by grinding yeast with sand and kieselguhr to destroy the cell structure and then submitting it, packed in cloths, to hydraulic pressure. The quantity of juice obtainable is very variable, but may amount to 250 ml. from 1 kgm. of pressed yeast. Buchner concluded that it contained a dissolved substance on which the fermentative power of yeast depended. This he called zymase. Other active cell-free preparations have since been obtained, of which " maceration juice " is an extract prepared by drying yeast at between 78° and 86° F and afterwards macerating it for 2 hours in water at 95° F. Zymin is a dry powder obtained by treating pressed yeast with about 6 times its weight of acetone or mixtures of alcohol and ether or alcohol and acetone. These preparations ferment sugar more slowly than living yeast and the smooth action of the enzymes is to some extent modified as a result of their separation from the yeast cells. The successive reactions are in some cases incomplete and various intermediate products accumulate instead of being transformed immediately they are produced. Evidence of the formation of intermediate products, which are quite undetectable in fermentations with living yeast, has been obtained by the use of various specific inhibitors which check the reaction at different stages.

(688) Phosphate in Fermentation.

Harden and Young[2] (1905) proved that the presence of phosphate was essential for the breakdown of sugar in fermentations with yeast juice. They isolated a hexose-diphosphate,

$C_6H_{10}O_6(H_2PO_3)_2$ and in 1914 Harden and Robison[3] obtained a hexose-monophosphate, $C_6H_{11}O_6(H_2PO_3)$, since when a number of analogous substances have been discovered. These are among the most significant intermediate products in fermentation and it is believed that "phosphorylation" is an essential preliminary stage in all biological processes in which hexose breakdown occurs. For example, in the conversion of glycogen to lactic acid in muscle action, in the decomposition of carbohydrates by bacteria, moulds and higher plants and in respiration, as well as in fermentation. Addition of phosphate does not accelerate normal fermentations, as it does those with yeast juice, the rate of which can be increased 20 times, bringing it up to about half that with living yeast. The reactions follow each other so rapidly with living yeast that direct proof of the intervention of phosphate has been difficult to obtain, but it is believed to be essential and some evidence of its occurrence has been obtained by Macfarlane.[4] It has also been found that arabinose and galactose, which are not fermentable by brewery yeast, are not esterified by phosphoric acid (Schoen and Elion[5]).

Harden and Young's original equations for the phosphate reactions in the preliminary stages of fermentation by yeast juice suggested a cycle in which a relatively small quantity of phosphate was able to esterify a large amount of sugar, but did not explain the nature of the change involved in the conversion of the very stable glucose to a labile form, capable of decomposition by the alcoholic enzymes. They held that the free phosphate entered into combination with the sugar to form hexose mono- or di-phosphate (equation 1), while one molecule of the hexose was decomposed to alcohol and CO_2 by alcoholic enzymes. The free phosphate is used up in this reaction, and very little can be found during active fermentation by yeast juice. It is therefore necessary to find the source of phosphate for its continuation. As fermentation slackens, there is a rapid rise in the amount of free phosphate at the expense of hexose-phosphates. This is represented by equation 2, according to which hexose-phosphate is hydrolysed by enzymes in the yeast juice. This reaction, or a similar change with the monophosphate, was held to proceed during the whole course of fermentation, continuously providing free phosphate to react with the excess of sugar, in accordance with equation 1.

$$(1)\ 2C_6H_{12}O_6 + 2PO_4HR_2 = 2CO_2 + 2C_2H_5OH + 2H_2O + C_6H_{10}O_4(PO_4R_2)_2$$

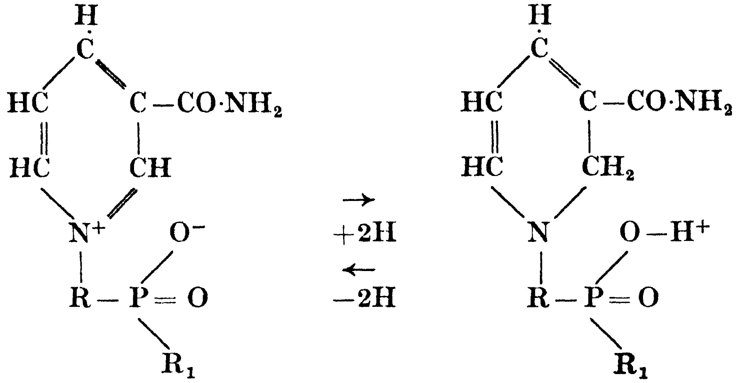
$$(2)\ C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2PO_4HR_2$$

Neuberg[7] suggested possible intermediate stages in the breakdown of hexose to the final products, alcohol and CO_2, but ignored the phosphate reactions. Methyl glyoxal, $CH_3{\cdot}CO{\cdot}CHO$, was held to be the first three-carbon compound formed. It was then converted to pyruvic acid, $CH_3{\cdot}CO{\cdot}COOH$, by an oxidation-reduction reaction. The pyruvic acid was supposed to be converted to acetaldehyde by removal of CO_2 by carboxylase, which Neuberg discovered. This last part of Neuberg's scheme is still accepted in explanation of the formation of alcohol, but methyl-glyoxal is not now believed to represent the first three-carbon compound and pyruvic acid has been shown to be the compound that gives up phosphate to more hexose, thus completing the cycle of reactions.

(689) Fermentation Enzymes and Coenzymes.

It is only by experiments with cell-free yeast preparations that it has been possible to distinguish the various enzymes concerned in fermentation. Zymase is not a single enzyme, but a complex with many activities. Harden and Young[8, 9] (1904) separated yeast juice in two fractions by ultrafiltration. That remaining on the filter consisted of protein, glycogen and dextrin, together with the enzymes. When dissolved in water, this fraction did not possess the power of fermentation which, however, was restored by addition of the dialysate to the solution. The activity of the enzyme fraction was thus shown to depend on the presence of another substance, the coenzyme. The latter was found to withstand boiling, whilst the enzyme fraction was rapidly inactivated by heat and could not afterwards be restored to activity by addition of coenzyme. This has been shown to comprise several constituents, of which coenzyme I and coenzyme II are the most important. The first was called cozymase by Harden and by Euler and has been identified by Warburg and Christian[10] as diphosphopyridine-nucleotide, a complex phosphate compound containing two molecules of the 5-carbon sugar ribose and the highly nitrogenous adenine.

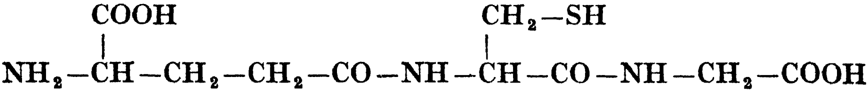
Oxidations and reductions are well known to occur in fermentations, thus acetaldehyde must be reduced to give alcohol, of which it is regarded as the precursor. Organic oxidations and reductions can take place either by gain and loss of oxygen or by loss and gain of hydrogen between one substance and another, but the transfer of hydrogen and not of oxygen appears to be the more important in biological oxidations, which are balanced by the reductions so that the system as a whole is neither oxidised nor reduced. A bridge between the two substances and motive power to transfer the hydrogen across it may be imagined. The former represents a substance that acts alternately as acceptor and donor of hydrogen, the latter is a specific enzyme or dehydrogenase. The intermediate function of the bridge is performed in fermentation, according to Warburg, by cozymase or coenzyme I, its pyridine or nicotinic-acid-amide group acting as hydrogen carrier. Coenzyme II is regarded as the phosphorylating coenzyme, and was identified as triphospho-pyridine nucleotide.



Cozymase Cozymase —H_2

(R = Ribose phosphate. R_1 = Adenosine)

Glutathion is another constituent of yeast to which active participation in oxidation-reduction reactions has been ascribed. It accounts for 0·6 to 1·0% of the dry weight of yeast and is a tripeptide, constituted by one molecule each of cysteine, glutamic acid and glycine, represented by



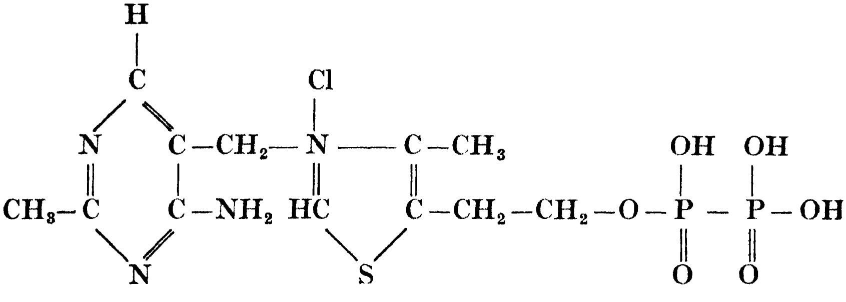
$$\mathrm{NH_2-\overset{\overset{\textstyle COOH}{|}}{C}H-CH_2-CH_2-CO-NH-\overset{\overset{\textstyle CH_2-SH}{|}}{C}H-CO-NH-CH_2-COOH}$$

The —SH group is the active part of the molecule, the transfer of hydrogen being represented by

$$2GSH \rightleftarrows GSSG + 2H$$

in which G indicates the remainder of the molecule. Its reducing power is another feature of its activity in living cells, supposedly stabilising the oxidation-reduction potential of the medium and cell contents.

Carboxylase is one of the most clearly defined enzymes of fermentation. Its action is inhibited at p_H 6·7, thus explaining the accumulation of pyruvic acid as the calcium salt, instead of its immediate decarboxylation, in fermenting sugar solutions to which calcium carbonate has been added. It also has been shown to be associated with a dissociable group which is removed from yeast by washing in alkali. This cocarboxylase was found by Lohmann and Schuster[11] to be phosphorylated vitamin B_1.



Cocarboxylase

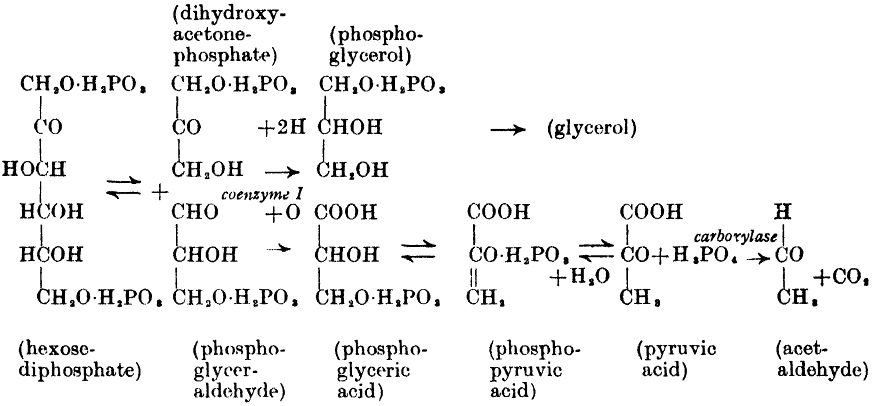
(690) The Mechanism of Fermentation.

The latest hypothesis of the sequence of reactions in fermentation, advanced by Meyerhof,[12] is based on investigations on glycolysis by muscle extract, as well as in fermentation, that resulted in the discovery of several intermediate products including a monophosphate of glyceric acid in fermentation (Nilsson[13]) and in experiments with muscle extract (Embden and Lagner[14]). The hypothesis also owed much to Embden's explanation of glycogen breakdown by muscle extract. Pyruvic acid is the first 3-carbon compound to appear free from phosphoric acid in the reaction sequence which, down to this stage, is the same in both processes of hexose breakdown. Pyruvic acid is then reduced to lactic acid in muscle extract and to a very small extent in fermentation, explaining its occurrence as a by-product in the latter, in which it is mainly converted by carboxylase to aldehyde and CO_2, the aldehyde being finally reduced to alcohol.

Meyerof divided the whole process into initial and stationary phases. The former ceases as soon as sufficient acetaldehyde is produced to carry on the stationary or continuous phase, in which the reactions are different. He convinced himself that the sugar breakdown occurred through hexose-diphosphate and not through the more readily fermented hexose-monophosphate and that the primary product of decomposition was the phosphoric ester of a triose, phospho-glyceraldehyde. He pictured the phosphorylation and activation of glucose as occurring in three stages,

$$\text{glucose-monophosphate} \rightleftharpoons \text{fructose-monophosphate} \rightarrow \text{fructose-diphosphate},$$

which was then enzymically converted, without the intervention of coenzyme, to two triose-phosphates, phospho-glyceraldehyde and dihydroxyacetone-phosphate. An oxidation-reduction reaction, involving cozymase (coenzyme I), converts these to phospho-glyceric acid and phospho-glycerol, from the latter of which the small quantity of glycerol produced in alcoholic fermentations may be supposed to be formed. The phospho-glyceric acid is then enzymically converted in two stages, the first involving intramolecular shift of the phosphate group from the third to the second carbon atom, the second giving phospho-pyruvic acid by loss of water. The phospho-pyruvic acid is then dephosphorylated and finally converted to aldehyde by carboxylase. The reactions, omitting the intramolecular changes, are represented by—



REACTIONS IN INITIAL PHASE OF MEYERHOF'S FERMENTATION SCHEME

The initial phase ceases when the small amount of aldehyde required to start the continuous or stationary phase has been produced. The more important second phase then continues in cycles until the end of fermentation. Glucose is esterified by the liberated phosphoric acid and then immediately broken down by enzymes to triose-phosphate, presumably phospho-glyceraldehyde. The latter provides the hydrogen necessary for the reduction of the acetaldehyde to alcohol, being itself oxidised to phospho-glyceric acid in a reaction involving dehydrogenase and coenzyme I.

$$\begin{array}{ccc} 2CH_2O(H_2PO_3)\cdot CHOH\cdot CHO & +\ 2O & 2CH_2O(H_2PO_3)\cdot CHOH\cdot COOH \\ & \longrightarrow & \\ & \text{coenzyme I} & \\ & \longrightarrow & \\ 2CH_3\cdot CHO & +\ 4H & 2CH_3\cdot CH_2OH \end{array}$$

The phospho-glyceric acid is then enzymically converted to phospho-pyruvic and pyruvic acids, as in the initial stage. A continuous supply of H_3PO_4 for esterification of more glucose, by intervention of coenzyme II and the necessary phosphatase, and of aldehyde for oxidation of phospho-glyceraldehyde by aldehyde-mutase in conjunction with coenzyme I thus becomes available and the cycle of reactions continues until all the glucose is used up or phosphate fails. The whole process is summed by the following equation in which a molecule of hexose-diphosphate appears on both sides to emphasise the fact that it is really this substance which is converted to alcohol and CO_2, glucose being the acceptor of phosphate. The hexose-diphosphate is the mother substance of triose-phosphate which reacts directly with cozymase, while glucose is being converted to diphosphate by way of monophosphate.

$$\begin{aligned}&1 \text{ hexose-diphosphate} + 2 \text{ glucose} + 2H_3PO_4\\ &= 2CO_2 + 2 \text{ alcohol} + 2 \text{ hexose-diphosphate.}\end{aligned}$$

(691) Hydrolysing Enzymes in Yeast.

The yeast cell is the seat of many other processes concerned with the hydrolysis of nutrient substances, synthesis of structural and reserve materials and respiration. Many of these reactions are due to enzymes of similar character to those dealt with in Chapter VIII. The fermentation of disaccharides is generally, though not universally, held to be dependent on the presence of the enzymes necessary to hydrolyse them to hexoses, namely sucrase, maltase and lactase. Enzymes of the amylase group synthesise or break down the yeast carbohydrates, glycogen, etc. Willstätter and Rohdewald[15] attributed the first stage of fermentation and production of active hexose to enzymes of this kind. They suggested that the sugar was first condensed in the yeast to glycogen, which was afterwards broken down to active glucose by an amylase. Smedley-Maclean and Hoffert[16] had previously obtained some evidence of the direct synthesis of glycogen from maltose.

The proteolytic enzymes required for the utilisation of nitrogenous nutrients and for the synthesis of yeast proteins must be present. Of these a proteinase, a polypeptidase and a dipeptidase have been detected and are believed to be concerned also in the autolysis of yeast that occurs when its normal activities are upset by temperatures above 77° F. (Section 685). Fat-splitting and -synthesising enzymes of the lipase group may be supposed to occur in the cells, with phosphatases and many others associated with different yeast constituents. At least four different phosphatases have been described (Vol. I, Section 134), and apparently take part in the phosphate metabolism in yeast and fermentation.

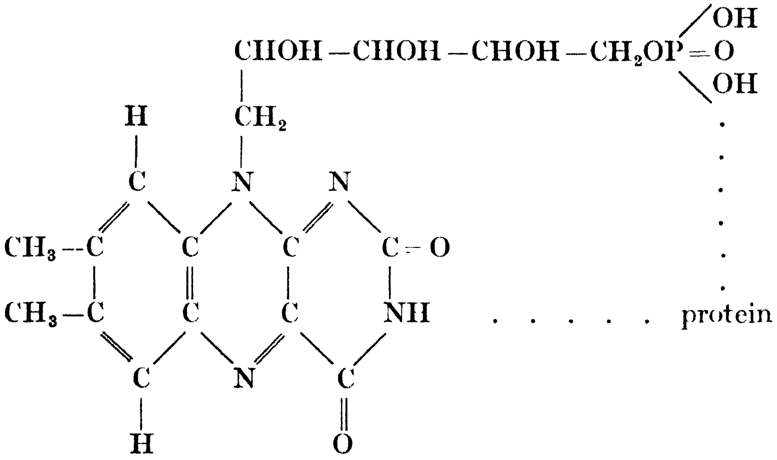
RESPIRATION

(692) Respiration and Yeast Growth.

The question of the part played by aeration in the metabolism of yeast raises a number of disputed points. Its practical aspects were dealt with in Section 563. There is no doubt that yeast growth can proceed in the almost complete absence of air, but it is greatly stimulated by aeration. Respiration produces much more energy than fermentation and is regarded as the main source of that required for the multiplication of yeast. It is, however, no longer thought necessary to correlate it with combustion, in which the direct intervention of oxygen is required to produce CO_2 and water. Reduction of alcohol production with increasing aeration is known as the "Pasteur effect," suggesting an antagonism between fermentation and respiration, but, according to Szent-Györgyi,[17] they "are intimately mixed and woven into one energy-producing system." Respiration takes the greater share in building up the brewery yeast crop, while fermentation breaks sugar down to alcohol, their respective shares in the total energy-production depending on the degree of aeration, the type of yeast and wort composition. In the extreme case of yeast manufacture with unlimited aeration, little or no alcohol appears.

Fermentation and respiration are both essentially dehydrogenation reactions. Oxidation in plant and animal cells results from the transfer of hydrogen from the substance being oxidised to another undergoing reduction. This can only occur through the activating influence of enzymes, with the aid of carrier systems capable of reversible oxidation and reduction. The enzymes appear to consist of an active, prosthetic group, coupled with a specific protein. Cozymase, for example, appears to take part in both dehydrogenase and reductase systems in fermentation. Coupled with one particular protein in the yeast cell, it is enabled to take hydrogen from phospho-glyceraldehyde which is thereby oxidised to phospho-glyceric acid, while the cozymase is converted to a reduced form. The reduced cozymase then gives up the hydrogen to aldehyde which is reduced to alcohol, the cozymase being reoxidised so that it can repeat the operations.

Respiration differs from fermentation in that the hydrogen is finally stabilised as water by combination with oxygen. Certain carrier systems are believed to be essential intermediaries in the process. Among these, cytochrome has long been known as an almost ubiquitous constituent of animal tissues and micro-organisms, in which it exists as a yellow haemo-protein. It was discovered in yeast by Keilin,[18] who believed that it accepted hydrogen from substrates under the activating influence of a specific dehydrogenase. Three components, known as cytochrome A, B and C, have been reported, the hydrogen being passed from one to another and finally combined with oxygen in presence of a special oxidase. Cytochrome occurs more abundantly in actively respiring bakers' yeast than in brewery yeast, and is not found in anaerobic bacteria.



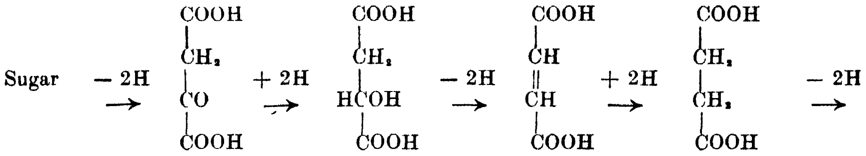
(Vitamin B_2 or Lactoflavin)

WARBURG'S "YELLOW ENZYME"

Other substances containing an active flavin group, which is yellow when oxidised and colourless in the reduced state, have also been discovered in yeast. Among these is Warburg's "yellow enzyme," consisting of the phosphate ester of lactoflavin or vitamin B_2, attached to a protein. The actual part played by these carriers in respiration is still in doubt, one suggestion being that coenzyme I or II may function as the dehydrogenase necessary to activate the hydrogen and convey it from the substrate to a flavo-protein which, in its turn, is reoxidised by the cytochrome-oxidase system.

The reactions involved in the earlier stages of fermentation and respiration are probably the same. Numerous suggestions have been made in regard to the point in sugar breakdown at which they diverge. It may be at the triose-phosphate, the reduced cozymase, after production of phospho-glyceric acid, yielding hydrogen to aldehyde, or through the cytochrome system, as the case may be. CO_2 might be supposed to arise in both through decarboxylation of pyruvic acid. Lactic acid may also be an important intermediary in respiration or the branching may occur at the hexose-phosphate stage, with diphospho-pyridine nucleotide as the coenzyme of fermentation and triphospho-pyridine nucleotide as that of respiration, hydrogen being transferred to the yellow enzyme.

According to Szent-Györgyi[17] respiration proceeds through a series of dicarboxylic acids, the hydrogen, after being passed along the series, under the influence of a specific dehydrogenase, is held to be dealt with by cytochrome, followed by the yellow respiration enzyme, eventually producing water.



$$\text{Sugar} \xrightarrow{-2H} \begin{array}{c}COOH\\ |\\ CH_2\\ |\\ CO\\ |\\ COOH\end{array} \xrightarrow{+2H} \begin{array}{c}COOH\\ |\\ CH_2\\ |\\ HCOH\\ |\\ COOH\end{array} \xrightarrow{-2H} \begin{array}{c}COOH\\ |\\ CH\\ \|\\ CH\\ |\\ COOH\end{array} \xrightarrow{+2H} \begin{array}{c}COOH\\ |\\ CH_2\\ |\\ CH_2\\ |\\ COOH\end{array} \xrightarrow{-2H}$$

(oxalo-acetic acid) (malic acid) (fumaric acid) (succinic acid)

(693) Summary.

There are few subjects in the realms of chemistry that have aroused such interest as the nature of fermentation. It is apparently such an ordinary and commonplace phenomenon, but the enormous difficulties encountered in tracing the manner in which sugar is converted to alcohol and carbon dioxide are revealed in the disputations that have raged around it during the last century. It is just over a hundred years since yeast was first declared to be a vegetable organism but forty years were spent in acrimonious dispute of that question. Then another twenty years passed before the means for reconciling the upholders of the chemical and vitalistic theories were discovered, although their quarrels had long since ceased.

Pasteur's definition of fermentation as life without air is valid in so far as fermentation is possible without air, but yeast contains the necessary enzymic apparatus for respiration and can only multiply to a limited extent if this is not brought into operation. The reproduction necessary for brewing cannot be secured without the energy derivable from respiration, but this side of yeast metabolism can easily be excited to too great a degree by excessive aeration. The main function of rousing is therefore regarded as the means whereby yeast is kept in suspension in the wort, the initial air supply usually being adequate for reproduction.

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CHAPTER XL

STABILITY OF BEER
OXIDATION-REDUCTION POTENTIAL
NON-BIOLOGICAL HAZES

AIR AND BIOLOGICAL STABILITY

(694) Respiration, Yeast Growth and Beer Stability.

The energy required by yeast for the synthesis of complex organic compounds from simpler substances lends great significance to the fact that the aerobic breakdown of glucose, associated with respiration, yields 20 times as many calories as anaerobic fermentation of the same quantity of sugar. Though many of the reactions involved in respiration may be explained by hydrogen transfer, supply of oxygen is essential for the final production of CO_2 and water. Only about a ninth of the energy liberated in anaerobic fermentation is utilised in yeast growth, and the cells must ferment about 50 times their weight of sugar to supply their energy requirements. The proportion utilised in synthesis can be greatly increased by continuous aeration and supply of nutrients at low concentration, as in yeast manufacture. Under the conditions of brewery fermentations, greater initial concentration of nutrients and limited oxygen supplies, the movement associated with aeration appears to be the important stimulating factor. Saturation of the wort with oxygen is, however, necessary for the reproduction required in normal fermentations.

The influence of oxygen on the behaviour of culture and wild yeasts is one of the most important factors in the stability of cask-conditioned beer. It was pointed out in Section 674 that no single factor could control the stability of beer, in so far as this depended on its resistance to bacterial growth. Complications arise from the varying physiological characters of different species and their reactions to stimulating or restricting influences. Yeast frets are much more common than bacterial defects, but identification of the causative organisms and application of preventive measures are even more seriously hampered by lack of information on the characteristics of species and races than in the case of bacteria. This applies to culture yeasts, as well as to those which are regarded as wild. Their individual reactions to stimulating and inhibiting factors are largely unknown. It appears, however, that variations occur in the degree to which their growth is stimulated by oxygen or inhibited by alcohol and other excretory products, in their bios requirements and, possibly, in the complexity of the carbohydrates and other substances which they are able to degrade. The stability of beer must therefore depend, to some extent, on the nature of the organisms, but in present circumstances it is necessary to consider it from a general standpoint.

Among the factors governing the stability of cask-conditioned beers are the original gravity of the beer and the quantity of alcohol and other inhibiting substances produced during primary fermentation, its attenuation or the quantity of carbohydrate, nitrogenous and other nutrients present at racking. Variations in the quantity of hop antiseptics and in p_H are of less significance than is the case with bacteria, but the quantity and physiological condition of the yeast, primary and wild, are of great importance. The activity of primary yeast may, for example, be influenced by its previous history in the fermenting vessel, and that of wild yeast may be quite small in the case of desiccated cells. The infection might, on the other hand, be virulent if it were due to cells already growing actively in a dirty cask or main. Culture yeast is usually more or less dormant at the close of primary fermentation, but any wild yeast present in the beer may be gaining in activity. Stimulation of yeast activity by aeration, in conjunction with agitation at racking and addition of primings, cannot fail to have important effects. There must be competition in practically all cases. The result will depend on which can most readily liberate energy by respiration and utilise it in growth.

Gentle stimulation of primary yeast produces normal cask condition, excessive activation leads to a primary yeast fret. If the culture yeast retains the dominance, it will rapidly absorb the available oxygen and a state of stability will be reached, without undue development of competing organisms, but if the quantity of culture yeast is very small or if its activity is greatly reduced, wild yeast, even if originally in relatively small quantity, will be activated and wild yeast fret supervene. This is the more likely to happen if the respiratory activity of the particular wild yeast is greater than that of the culture yeast. Several references to the influence of oxygen on yeast growth have been made, particularly in Sections 563, 619 and 650.

(695) Effects of Air on Bottled Beer.

One of the most important functions of conditioning is the removal of dissolved air from beer and free air from the tanks, and emphasis must be laid on the prevention of its absorption at every stage in the bottling process. Some of the detrimental effects produced by solution of traces of oxygen in bottled beers are summarised below. These are particularly liable to be noticed with non-deposit beers, oxygen being absorbed by the slow growth of yeast in bottle-conditioned beers, to an extent that is usually sufficient to prevent them. Air may, however, cause the development of a film of *Mycoderma* in infected bottles. The effects of air are usually rather slow and progressive during storage, but they may be detected by :

(1) Deterioration of flavour.
(2) Increase in colour.
(3) Intensification of " pasteurisation flavour."
(4) Acceleration and increase of haze production.
(5) Reduction of biological stability.

(696) Biological Instability of Bottled Beer.

Among the questions which arise when an unusually early or active development of primary yeast, wild yeast or bacteria occurs in filtered bottled beer are (*a*) how came the invading organisms to be present in the bottles and from what source were they derived, and (*b*) is their growth due to an inherent instability in the beer, some defect in composition that causes it to be a more than usually favourable nutrient medium or to an exceptional virulence or activity in the invader. An answer to the first question can generally be found by examination of the bottles and every part of the plant with which the beer has been in contact. Samples are obtained by rinsing all these with a little sterile water and cultivation in suitable liquid and solid nutrient media. Tanks, casks, bottles, mains, valves, carbonator, filter pulp, and every part of the bottling machine and the air used for compression all come under suspicion. Samples of the beer are taken in sterile bottles at every point in the bottling line from which a sample can be taken. The air in the bottling department should also be examined by exposure of plates and other methods, but this is the least likely source of contamination. If such actually exists it is much more likely to be in the plant. The bottle washer is very likely to be the culprit and would be detected by examination of the bottles, or some rather inaccessible valve or part of the filler has been neglected. In any case the remedy is obvious.

Rapid growth of micro-organisms, however, not infrequently occurs without any well-marked external cause. The composition of the beer naturally comes under suspicion in such cases. Does it contain too much fermentable sugar, has the attenuation been defective ? The answer to such questions may prove unsatisfactory. A beer that might be expected to encourage the growth of yeast or bacteria proves stable or another that is well attenuated is unstable. It is impossible, at present, fully to explain these occurrences, but their virulence appears to be due to conditions existing in the beer, rather than to special activity of the microorganisms. Some of the factors that encourage or restrict yeast growth in bottled beer are still unknown, but oxygen is probably the most important stimulating agent. De Clerck[1] put the factors responsible for yeast development in the following order: (1) race of the yeast, (2) presence of air, (3) presence of fermentable sugar, (4) unknown resistance factors in beer, and regarded the original number of cells and their physiological state or age as of less importance.

Light beers are generally less stable than strong, and microorganisms may grow better in them in the absence of air than in strong beers in its presence. De Clerck gave the following data in illustration of the influence of oxygen on stability, as measured by the period during which a certain beer remained bright and before yeast growth could be detected when incubated at 77° F.

TABLE 243.—INFLUENCE OF AIR ON YEAST DEVELOPMENT IN BOTTLED BEER
(J. DE CLERCK)
(INCUBATED AT 77° F.)

Ml. Oxygen per litre	First appearance of Yeast growth, days
nil	15
1·5	11
5	7
20	6

The effect of oxygen is particularly marked with yeasts that cause frets. The practice of priming British beers may alter the situation as regards the relations between air and stability. Little evidence is available on this at present, but there is no doubt that air is a most important factor. The effect of air on bacteria depends on whether they are aerobic or anaerobic and, if facultative, also on the presence or absence of fermentable sugar. De Clerck's experiments were carried out with lager beer and the tendency is for greater aeration in bottled top fermentation beers, which may also have stronger powers of resistance on account of their lower p_H and higher hop rate. They show, however, that every possible step should be taken to minimise the quantity of air present.

The amount of air present in bottled beer is liable to vary considerably from bottle to bottle of the same filling, and this involves an obvious difficulty in determining whether the growth of yeast is related to the oxygen content or not. If the oxygen is determined, the sample itself is lost for purposes of stability estimation. It was this that led De Clerck[2] (1933) to investigate the possibility of using oxidation-reduction indicators for obtaining an idea of the degree of aeration in samples that were also tested for stability. The method proved successful and led to the investigation of the influence of oxygen in other directions. Methods based on this discovery are now used for control purposes, and it is therefore necessary to describe in a simple manner some of the principles on which they depend. Reference should be made to De Clerck's original papers, to special text-books or such special publications as those by Hewitt[3] and Glasstone[4] for fuller information.

OXIDATION-REDUCTION POTENTIAL

(697) Oxidation-Reduction Systems.

Reference was made in Section 689 to the significance of oxidation-reduction reactions in fermentation, by which was meant chemical changes that involved oxidation of one substance and simultaneous reduction of another. These are so important in biochemistry and in the activities of living things that it is necessary to have some means of measuring the oxidising or reducing properties of a substance and the progress of oxidation-reduction processes. These do not necessarily consist in the transfer of oxygen or hydrogen from one substance to another. Oxidation and reduction have a wider significance which, according to the electronic conception of atomic structure, is associated with the transfer of electrons from the oxidised to the reduced substance. This can be most readily visualised by consideration of a very simple example, such as the " oxidation " of a ferrous salt to a ferric salt and " reduction " in the reverse direction. Atoms are regarded as composed of a nucleus, positively charged with electricity, and a number of electrons, each bearing a unit negative charge. The number of electrons concerned differs with different elements, but the positive charge on the nucleus is numerically equal to the sum of the unit negative charges on the electrons, so that the element is electrically neutral. A similar state of neutrality exists in a salt, but when this is ionised in dilute solutions, one or more negatively charged electrons are supposed to pass from the metal to the other ion. As a result of the loss of negative charges, the metal ion becomes positively charged, while the other ion becomes negatively charged on account of their acceptance. The ionisation of ferric chloride is thus represented by the transfer of three unit negative charges or electrons from iron to chlorine and the resulting charges on the ions would be represented by :

$$FeCl_3 \rightarrow Fe^{+++} + 3Cl^-$$

The oxidation of ferrous chloride to ferric chloride, when both are ionised in dilute solution, would similarly be represented by :

$$Fe^{++} + 2Cl^- + Cl \underset{\text{reduction}}{\overset{\text{oxidation}}{\rightleftarrows}} Fe^{+++} + 3Cl^-$$

This means that the ferrous ion on being oxidised to the ferric state loses one electron and becomes more highly positively charged. The electron cannot exist in the free state and is taken up by chlorine. This reaction is reversible, as indicated by the arrows, and provides an example of the very important *reversible oxidation-reduction processes.* By reading the equation in the opposite direction, it will be noted that reduction of the ferric ion is represented by the gain of a negatively charged electron, whereby its positive charge in the ferrous state is reduced. This may be expressed by :

$$Fe^{++} \underset{\text{reduction}}{\overset{\text{oxidation}}{\rightleftarrows}} Fe^{+++} + \text{electron}$$

The points to notice are: (1) Oxidation cannot take place without reduction or reduction without oxidation. (2) The oxidised substance is represented as more highly positively charged than the reduced substance, owing to the loss of negative charges. (3) Such reactions are frequently reversible and the substances concerned constitute a *reversible oxidation-reduction system.*

(698) Oxidation-Reduction Potential.

It would be expected that the transfer of electrons during the course of oxidation-reduction processes could be detected by methods that reveal the passage of an electric current. This is actually the case. If an electrode of some metal which is not attacked by the solution in question (platinum or gold is generally used) is placed in a reversible oxidation-reduction system, a potential, dependent on the relation between the concentrations of the oxidised and reduced forms, is set up at the electrode. It is not possible to measure a single potential difference at an electrode as this constitutes only a half-cell, but if the circuit is completed by including a standard half-cell, the electromotive force of the completed cell due to the difference of potential between the two electrodes may readily be measured. The second half-cell to which electrode potentials are referred is the normal hydrogen electrode, which is taken as the standard of reference. Electrode potentials referred to this standard are measured in volts and designated E_h. If E is the actual potential set up at the oxidation-reduction electrode, $E_h = E -$ potential of the normal hydrogen electrode in the same solution.

The value of E_h in volts can be calculated from the general electrode equation when the system is thermodynamically reversible by :

$$E_h = E_o + \frac{RT}{nF} \log_e \frac{[Ox]}{[Re]}$$

in which E_o is a constant, characteristic of the system, and represents the electrode potential when the system is 50% oxidised and 50% reduced. T is the absolute temperature $= (273 + t)$ °C. By introducing the known values of R (8·31507 volt coulombs) and F (the Faraday = 96500 coulombs) and converting to ordinary logarithms by means of the factor 2·302585, the equation becomes :

$$E_h = E_o + \frac{0{\cdot}0002\ T}{n} \log \frac{[Ox]}{[Re]}$$

For a system at constant p_H and 17° C. in which two electrons are concerned this becomes :

$$E_h = E_o + 0{\cdot}029 \log \frac{[Ox]}{[Re]}$$

E_h *is the oxidation-reduction potential* of the system in volts, referred to that of the normal hydrogen electrode at the same p_H. It is sometimes abbreviated to redox potential and may be regarded as the force that brings about the transfer of electrons. It becomes equal to the standard potential of the system when the molecular concentrations of the oxidised and reduced forms are equal, because $\frac{[Ox]}{[Re]}$ then = 1 and its logarithm = 0. It is necessary to introduce the restriction "at the same p_H," because the potentials of the hydrogen electrode and of other systems vary at different p_H values and not necessarily in parallel. The oxidation-reduction potential can therefore only be characterised fully by giving both E_h and p_H, the potential of the normal hydrogen electrode being known at different p_H values.

In many organic systems, however, the potential due to hydrogen ions varies lineally with the p_H and in parallel with that of the hydrogen electrode. In such cases

$$E_h = E_o + 0{\cdot}029 \log \frac{[Ox]}{[Re]} - 0{\cdot}058\, p_H$$

and, when $[Ox] = [Re]$, $E_h = E_o - 0{\cdot}058\, p_H$.

This holds with the quinhydrone system, which divides in solution into equimolecular proportions of the reduced and oxidised forms, hydroquinone and quinone,

$$C_6H_4(OH)_2 \rightleftarrows C_6H_4O_2 + 2H^{\cdot} + 2e$$

The potential at an indifferent electrode immersed in quinhydrone solution, thus depends on E_0, which is 0·704 volt at 18°–20° C. and the p_H of the solution. Actually the oxidation-reduction potential is measured when the quinhydrone electrode is used in hydrogen ion determinations, and p_H is calculated from it. 0·437 is the potential (E_h) of the standard half-cell described in the next Section (Cole's standard acetate solution, p_H 4·63 + quinhydrone). This E_h value may be obtained from the equation

$$E_h = 0{\cdot}704 - 0{\cdot}058\, p_H, \text{ whence } E_h = 0{\cdot}704 - 0{\cdot}058 \times 4{\cdot}63$$

(699) Potentiometric Measurement of Oxidation-Reduction Potential of Beer.

The oxidation-reduction potential of beer is usually measured against that of some such electrode as the quinhydrone or calomel. Some forms of the glass electrode are also satisfactory. Their potentials in reference to the normal hydrogen electrode are known and the potential set up at the oxidation-reduction electrode can consequently be calculated. Thus the measured potential, when a quinhydrone electrode is used, is the difference between the potential of the quinhydrone electrode and that set up at the oxidation-reduction electrode. The former, in reference to the hydrogen electrode, is 0·437 volt, if sodium acetate-acetic acid buffer solution at p_H 4·6 is used. The oxidation-reduction potential of the liquid under test is then given by

$$E_h = 0{\cdot}437 - E$$

Potentiometric measurements are delicate. They are upset by dissolved oxygen and the readings are readily disturbed by electric currents. Objections in respect of contact with air and adsorption of oxygen on the electrode are, however, removed in the apparatus and process described by stirring during washing

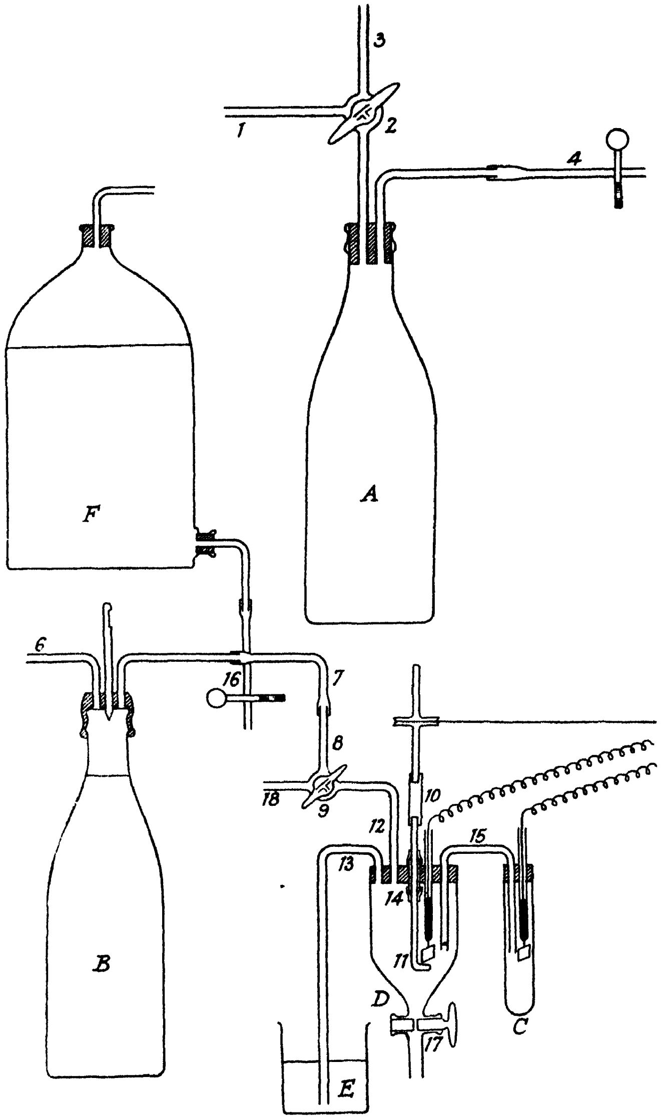


Fig. 218
APPARATUS FOR DETERMINING OXIDATION-REDUCTION POTENTIAL OF BEER
(Dixon and Hill)

of the electrode. Electrometric methods devised to eliminate these disturbing factors are no doubt more accurate than the simpler colorimetric tests dealt with in following Sections and, with improved apparatus, will continue to provide the standard of reference for determination of the oxidation-reduction potential of wort and beer in terms of E_h and p_H.

The apparatus shown diagrammatically in Fig. 218 was designed by B. Dixon and A. M. Hill and consists of a small vessel (D) in which the beer is placed with every care to avoid contact with air. This is effected by carrying out all operations in CO_2. A small square of thin gold foil, dipping into the beer, constitutes the oxidation-reduction electrode, the potential of which has to be measured. The cell also contains a stirring device to facilitate attainment of a constant potential. (C) is the second half-cell, a quinhydrone electrode which serves as the standard electrode against which the potential difference has to be measured. Connection is made between the liquids in the two half-cells by means of the bent tube (15) filled with agar jelly containing potassium chloride. This salt bridge ensures electrical contact without any syphoning of liquid from one cell to the other. The electrodes are then connected by wires through a potentiometer. This consists essentially of a dry battery with a variable resistance in circuit and a galvanometer by which the passage of a current can be detected. The potential at the oxidation-reduction electrode is lower than that at the quinhydrone electrode. The difference is measured by adjusting the resistance in the circuit until no current passes between them as indicated by the galvanometer needle pointing to zero. The reading on the dial then gives the difference in E.M.F. in millivolts between the quinhydrone and oxidation-reduction electrodes. If this was 238 millivolts and the difference between the potential of the quinhydrone electrode and the normal hydrogen electrode is 437 millivolts, the oxidation-reduction potential of the beer would be given by $437 - 238 = 199$ millivolts.

The following procedure is adopted for making a determination with beer in a crown-corked bottle. The electrode vessel D is filled with air-free distilled water from the reservoir F, and C with buffer solution at p_H 4·63, saturated with hydroquinone. The sodium acetate-acetic acid buffer employed for diastatic activity determinations is used. A rubber cap, fitted with two glass tubes and a steel needle, is pressed over the top of the bottle, and one of the tubes, 7, is connected to tube 8 of the apparatus. A rubber tube, connected to a source of CO_2, is now fastened to tube 6, and a stream of CO_2 blown through the rubber cap, through tubes 7 and 8, and out to the atmosphere at 18. After a minute or so, the crown cork is pierced by the steel needle, and the latter is then partly withdrawn. The water is blown from the electrode vessel by turning tap 9 until CO_2 commences to bubble through the water seal, E, then slowly turning tap 17. When the electrode vessel is empty, it is filled with the sample by inverting the bottle, and pinching the rubber connection to CO_2 at 6. If there is not sufficient pressure in the bottle to fill the electrode vessel completely, a little more CO_2 may be bubbled in from 6. The electrode vessel is usually washed out two or three times with the sample, using the stirrer to obtain maximum effect. When the sample is ready and the apparatus connected to the potentiometer, the stirrer is again used to hasten attainment of a constant potential.

A sample of beer is obtained from a conditioning tank by means of the bottle A. This is first completely filled with distilled water. Tube 1 is then connected to a source of CO_2, which is first blown out at 3. The bottle is then inverted, and the tap turned so that CO_2 bubbles into the bottle, when 4 is opened and all the water is removed. The clip 4 is then closed and tap 2 turned, so that the CO_2 again escapes at 3. 1 is now connected to the sample cock of the tank, and some of the beer allowed to run out at 3. The tap 2 is then turned, so that the beer commences to run into the bottle, when clip 4 is opened until the bottle is full. 4 and 2 are then closed. The operations for removing the beer from the bottle to the electrode vessel are similar to those described for the crown-corked bottle.

(700) The r_H Scale.

Oxidising and reducing agents may be arranged on a scale and graded according to their activities in a manner analogous to that adopted for acids and alkalies on the p_H scale. The basis of the scale is a hypothetical hydrogen pressure (P) at the oxidation-reduction electrode determined by E_0 and $\frac{[Ox]}{[Re]}$, which can be used to express the oxidation intensity of the system. The relation between the oxidation-reduction potential, p_H and the hydrogen pressure is given by:

$$E_h = 0{\cdot}0002\ T\left(\log\frac{1}{P} - 2\ p_H\right)$$

Mansfield Clark and Cohen (1923) used the symbol r_H for the negative logarithm of the hydrogen pressure, that is for $-\log P$ or $\log\frac{1}{P}$. The relation in a system at 17° C. is then:

$$E_h = 0{\cdot}029\ (r_H - 2\ p_H)$$

$$r_H = \frac{E_h}{0{\cdot}029} + 2\ p_H$$

From this it will be seen that the higher the value of r_H, the greater is the oxidising intensity of the system.

The meaning of the hydrogen pressure and its relation with potential will be appreciated from consideration of the hydrogen electrode as a type of oxidation-reduction system in which the oxidised form is represented by hydrogen ions and the reduced form by gaseous hydrogen. The concentration of the latter is a function of the hydrogen pressure, which is 1 atm. in a normal hydrogen electrode. The potential of a hydrogen electrode, with the gas at pressure P, is given by :

$$E = \frac{R\,T}{2\,F} \log_e \frac{1}{P} + \frac{R\,T}{F} \log_e [H^+]$$

The reversible potential of other oxidation-reduction systems, in which the reduced form differs from the oxidised by two atoms of hydrogen only, is given by :

$$E = E_o + \frac{R\,T}{2\,F} \log_e \frac{[Ox]}{[Re]} + \frac{R\,T}{F} \log_e [H^+]$$

Comparison of these two equations shows that an oxidation-reduction electrode of the type considered may be regarded as a hydrogen electrode with gas at a pressure determined by E_o and $\frac{[Ox]}{[Re]}$. Since these quantities determine the oxidation intensity of the system, this hypothetical pressure (P) may be expressed in terms of a symbol representing log $\frac{1}{P}$. This is r_H as previously defined. No such pressure of hydrogen gas can be measured in systems containing weak reducing agents, but hydrogen may be supposed to exist in them along with hydrogen ions and may be held to accumulate at the electrode until a partial pressure is reached, which is in equilibrium with the system and the latter is neither oxidised nor reduced. For this reason it was referred to as a hypothetical hydrogen pressure.

Use of r_H makes the tacit assumption that there is a simple relation between E_h and p_H. In many cases this does not hold and Clark now insists that the use of r_H should be abandoned and that all systems should be characterised by E_h and p_H. The curves for variation of the potential at an oxidation-reduction electrode immersed in dilute solutions of many dyes is, however, nearly parallel with that for the normal hydrogen electrode and it seems permissible to retain the r_H scale, on account of its convenience, when these are used for determination of the oxidation-reduction balance of beers, as described in the next Section. It should, however, be noted that the fundamental requirements on which the conception of r_H was based are not satisfied by beer. The oxidation-reduction systems existing in it, and the manner in which r_H varies with the electrode potential and p_H for the systems involved are unknown. Retention of the r_H scale for beer must therefore be regarded as empirical, and only justified in so far as the results are proved to have useful applications. It is not to be regarded as an easily measurable property, having definite significance in respect of beer stability. Determinations have, nevertheless, afforded a means for measuring the effects of air or oxygen on the biological and non-biological stability of beer, with a precision which had previously been lacking. The results have emphasised the immense practical importance of avoiding contact with air, and led to technical advances of great value.

(701) Oxidation-Reduction Indicators.

Many dyes yield colourless leuco compounds on reduction and the leuco compounds revert to the coloured substances on oxidation. A mixture of the coloured and colourless compounds thus constitutes a reversible oxidation-reduction system, its depth of colour depending on the proportions of the oxidised and reduced forms. The change from full colour to colourless takes place over a comparatively short r_H range, which is known for many dyes, and usually corresponds with a potential fall of about 0·1 volt. The change in colour intensity is also proportional to the fall in r_H over this range. Since the potential of a mixture at half colour, consisting of 50% dye and 50% leuco compound, varies with the p_H of the solution, it is usual to refer to the standard potential of the dye at a given p_H value. This is expressed as E'_0. It is $+ 0{\cdot}011$ volt at p_H 7 and 30° C. for methylene blue, for example. If a small quantity of such a dye solution is added to an oxidation-reduction system, an equilibrium is reached at which the potentials of the latter and of the dye system are identical. If the quantity of the dye solution is relatively small and the resistance of the other system to change of potential is considerable, the dye will adjust itself until its potential becomes the same as that of the solution in which it is placed. This potential can be judged from the colour intensity of the mixture, and the dye thus acts as an *oxidation-reduction indicator*, over the range of r_H or E_h within which it changes from full colour to colourless. Its useful range is actually rather shorter than this, as it is difficult to judge changes in colour intensity when the colour is nearly full or nearly discharged. Some data for a few useful indicators are given in the following Table.

TABLE 244.—OXIDATION-REDUCTION INDICATORS

	E'_0 (volt) at 30° C.		Approximate r_H range
	p_H 4·5	p_H 7·0	
2 : 6-dichlorophenol-indophenol ..	0·405	0·217	20–22
o-cresol-indophenol	—	0·191	18–20
1-naphthol-2-disodium sulphonate-indophenol	—	0·123	16–18
Lauth's violet (thionine) .. .	0·170	0·063	14–16
Cresyl blue	0·175	0·047	14–16
Methylene blue	0·138	0·011	13–15
K-indigo-tetrasulphonate	0·095	-0·046	11–13
K-indigo-trisulphonate . .	0·059	0·081	10–12
K-indigo-disulphonate .. .	0·018	-0·125	8–10

Under the assumption that r_H is independent of p_H, these dye solutions could be used as indicators for r_H at any p_H value. This is not quite true, but the curves for variation of potential with p_H for many of them and for the hydrogen electrode are sufficiently nearly parallel to make the assumption reasonably valid for many practical purposes. The r_H represented by a half-decoloured indicator solution at 30° C. and p_H 4·5 or 7·0 can be calculated by substituting the E'_0 values given in the Table for E_h in the equation $E_h = 0{\cdot}03\ (r_H - 2p_H)$. This would give r_H 13·6 and 14·4 at p_H 4·5 and 7·0 for methylene blue, for example.

(702) Poising Effect.

The oxidation-reduction potential, like p_H, is a measure of intensity level and not of capacity, since it depends on the ratio of the oxidised and reduced forms and not on their quantity. Thus, a system containing 70% oxidant and 30% reductant will have the same E_h, whether the total concentration is 1% or 10%, but the resistance to oxidation or reduction will be 10 times greater in the latter case than in the former. This capacity to resist oxidation or reduction is known as "poising" and it is in many respects analogous to the buffering of acid-base systems. Mendlik,[5] who first applied potentiometric methods to measurement of the oxidation-reduction balance of beer, made an electrometric titration curve for beer with an oxidising agent and found that the beer was not poised against variations in oxidation-reduction potential to more than a very slight extent. This explains why the smallest contact with air has such a great effect on its oxidation-reduction potential and why it is essential to carry out all determinations of E_h or r_H of beer in an atmosphere of CO_2 or nitrogen and with scrupulous care to avoid the slightest contamination with air. It is only permissible, for the same reason, to add a very small quantity of an oxidation-reduction indicator to beer. Otherwise the oxidation-reduction level of the beer would tend to adjust itself to that of the indicator. This also means that an indicator for use with beer must have an intense colour, contrasting with that of the beer. This practically excludes all indicators that are not blue. The first three in Table 244 are blue in alkaline solution, but red in beer and consequently their decoloration is very difficult to see in beer. Lauth's violet is violet at all p_H values, the other indicators given are blue at all p_H values. These have proved very useful with pale lager beers, in which a very small quantity gives a green colour. The position is different with British beers, firstly because their r_H is generally higher than that of lager beers and no suitable indicator is available. Secondly, their colours are usually darker than that of pale lager beer, making difficult the use of the blue indicators when the r_H is low enough to come within their ranges. Potentiometric methods must consequently usually be adopted for British beers and are to be advised in all cases on account of their greater accuracy.

(703) Oxidation-Reduction Balance of Beer.

It is well known that yeast exerts a reducing effect during fermentation. Even filtered beer exhibits a slow reducing tendency, but it is not known to what substances its reducing power is due. Among suggestions made are that it may be due to sugars or to reducing substances produced from them, to hop resins or to free-SH groups formed in the protein-tannins during wort boiling (Hartong[6]). For all practical purposes, it is the oxygen of any air present in solution that decides its oxidation-reduction potential and it is as a means of estimating the degree of aeration that determination of the r_H of beer has found its most useful practical application. It should, however, be noted that it cannot give an accurate measure of the quantity of air or percentage of oxygen present. What the determination of oxidation-reduction potential can do at present is to indicate whether the air content of beer is sufficient to lead to reduction of its stability, both biological and colloidal.

Since the growth of micro-organisms depends to such an extent on the quantity of air in the beer, determination of E_h or r_H provides a convenient method for forecasting its biological stability. Tests at the filling machine show that beer is usually aerated too much at this stage and that considerable variations occur in bottles filled in one batch. During fermentation the r_H can go down to between 9 and 10, but the r_H increases considerably every time the beer is moved and air gains access. De Clerck found that lager beer from storage tanks had an r_H of about 10–11, which was raised to 17–18 in the bottles. The reducing power of the beer then gradually lowers the r_H to its original value of 10–11, if the quantity of air absorbed at filling is not too great. He found that, in most cases, yeasts did not grow or only developed to a slight extent in bottled lager beers that rapidly attained an r_H below 13, that is if methylene blue was decoloured. Laufer[7] obtained values between r_H 11·5 and 16·6 for American bottled beers.

No determinations of the oxidation-reduction potentials of British beers have been published. Those given in Table **245** were privately communicated to the writer by B. Dixon, who agreed with De Clerck's findings that yeast development in bottle was very unlikely when the r_H fell to about 13. Lower values are reached in bottle-conditioned beers and can be attained in the conditioning and cold storage tanks, if every care is taken to exclude air. Transfer from one tank to another, however, always leads to increase in r_H and this usually becomes very high at the bottling machine. Dixon also confirmed the relation between oxidation-reduction potential and colloidal stability. Haze is much more likely to be formed or to be formed in a shorter time when the r_H is comparatively high.

TABLE 245.—OXIDATION-REDUCTION POTENTIALS OF BRITISH BEERS (B. DIXON)

(r_H CALCULATED FROM $E_h = 0{\cdot}03\ (r_H - 2p_H)$)

Beers	Time in bottle	E_h Milli-volts	p_H	r_H	
1 Bottle-conditioned	14 days	177	4·23	14·3	Different beers
2 ,,	1 month	162	4·00	13·4	
3 ,,	2 months	98	4·08	11·4	
4 Non-deposit	7 days	287	4·09	17·7	Different bottlings of same beer
5 ,,	13 ,,	214	4·05	15·2	
6 ,,	19 ,,	152	4·12	13·3	
7 ,,	0 ,,	332	4·32	19·7	
8 ,,	0 ,,	324	3·80	18·4	Same beer
9 ,,	3 weeks	305	3·65	17·5	
10 Imported lager	—	313	4·15	18·7	
11 ,,	—	263	4·58	17·9	

The figures in Table **246** show that the oxidation-reduction potential at racking is low and may be still lower after conditioning in a tank. A small rise inevitably occurs if the beer is moved to a second tank for chilling, even though every precaution is taken to minimise air contact. A small increase also occurs during carbonation, owing to the presence of air in the CO_2, but the biggest rise takes place at bottling, with a further rise as air is absorbed from the neck of the bottle. The slow reducing action of the beer causes sufficient fall after this to ensure its stability, if the original r_H was not too high.

TABLE 246.—OXIDATION-REDUCTION POTENTIALS DURING STORAGE (B. DIXON)
(LIGHT PALE ALE)

	Eh Milli-volts	p_H	r_H	
A. Racking from F.V.	138	4·08	12·8	Bottled from " Redox " tank after 10 days.
Conditioning tank, before carbonation	121	4·08	12·2	
Conditioning tank, after carbonation	127	4·08	12·4	
After bottling	184	4·08	14·3	
2 days after bottling	196	4·08	14·7	
B. Racking from F.V.	138	4·10	12·8	Same beer in two-tank system.
Before moving to 2nd tank	123	4·10	12·3	
After moving to 2nd tank ..	147	4·10	13·1	
After carbonating	156	4·10	13·4	
After bottling	216	4·10	15·4	
2 days after bottling	273	4·10	17·3	
C. From bright beer tank ..	210	4·10	15·2	Same beer to bright beer tank.
After bottling	237	4·10	16·1	
2 days after bottling	300	4·10	18·2	

Great variation exists in the relation between the stability of British beers and their r_H values. Dixon has found that the same figure does not apply as a criterion in every brewery. In many cases stability is quite good, although comparatively high r_H values are obtained. The poise of the beer affects the r_H value to some extent and, as relatively little is known about poise in connection with beer, the effects of brewing materials on r_H are still to be investigated. The protein compounds of wort and beer will, no doubt, be found to play a part in this, and the hop rate seems bound to be important. De Clerck's[1] recent work is of great interest in connection with the significance of variations in the r_H values of different beers. It seems to offer a reasonable explanation, by pointing out that some yeasts are less affected by air than others In the present state of uncertainty in regard to the strains used in British breweries, it seems that what appears at the moment to be confusing, and to some extent contradictory, will have to await a classification of the yeasts in use. It follows that, in comparing the r_H values, not only must the method of determination be considered, but also the composition of the brewing materials and the strain of yeast used. The optimum r_H for stability of a particular beer depends on them, but the fact remains that it is important for the beer to reach its lowest r_H value, and the aim should be to utilise all means which circumstances permit for keeping it at the lowest attainable figure.

(704) Colorimetric Determination of the r_H of Beer.

The method originally adopted by De Clerck to determine the r_H of freshly bottled beer is useful for control purposes with pale beers. Sterilised solution of methylene blue (0·5 gram per litre) is added at the rate of 1 in 300,000 to clear glass bottles before they are filled and crowned in the usual way. Other indicators may also be used. Those which lose colour below r_H 17 retain their colour in beers of high air content. Those which change between r_H 17 and 11 lose colour in beers of low air content, at a rate depending on the amount of air in the beer and the transition range of the indicator. Lauth's violet may decolour rapidly, methylene blue may require a few days at laboratory temperature, while indigo-disulphonate may never lose its colour. If care is taken to exclude air by working in CO_2, Lauth's violet and methylene blue should be decoloured immediately by beers that are practically air-free. Any reducing tendency shown by the sample should show up within 3 hours and the final result be reached within 24 hours. The slow reaction that takes place after this may be disregarded.

Laufer[7] employed a more precise method in which tests are made in triplicate with three indicators, thionine, methylene blue and indigo tetra-sulphonate. Dye solutions of the following concentrations are used for pale beers.

TABLE 247.—OXIDATION-REDUCTION INDICATORS FOR BEER TESTS

	ml. 0·1% dye in 100 ml. beer	ml. 0·1% dye in 20 ml. beer	ml. 0·01% dye in 20 ml. beer
Thionine, cresyl blue or methylene blue ..	0·1	—	0·2
Indigo tetra-sulphonate	0·5	0·1	—
Indigo tri- and di-sulphonate	0·25	0·05	—

The tests are made in 100-ml. bottles or 20-ml. test tubes. Precautions must be taken to avoid contact with air when sampling from casks or tanks. Laufer used an Erlenmeyer flask, fitted with rubber bung and two glass tubes to which lengths of rubber tubing, closed with pinchcocks, were attached. One tube reached to the bottom of the flask, the other just through the bung. The flask is filled with CO_2 and kept in ice. Beer is allowed to enter through the long tube.

The necessary quantity of indicator is first added to the test tube or 100-ml. bottle, in which the air is then replaced by CO_2.

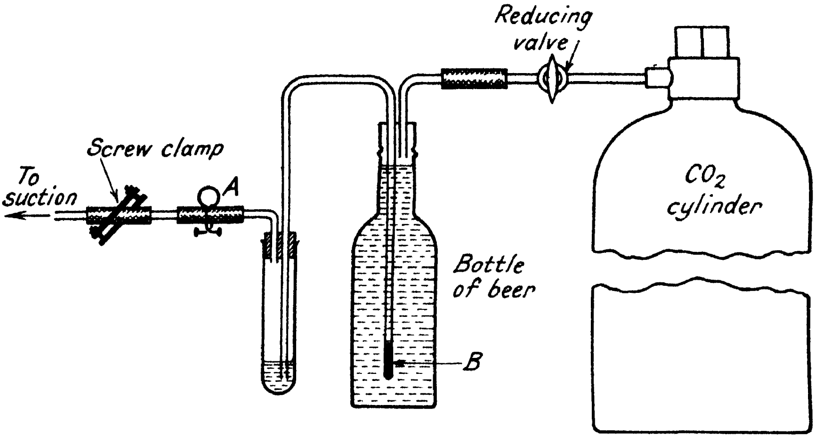


Fig. 219

FILLING TEST CONTAINER FOR r_H TEST (S. LAUFER)

The test containers are filled with beer from the Erlenmeyer flask or from a bottle by suction, as shown in Fig. 219. The bottle of beer to be tested is chilled, opened and a slow stream of CO_2 passed on to the surface of the beer. The glass plug from tube **B** of the test container is removed, and the tube immediately inserted in the sample. Tube A is attached, through a safety flask, to a vacuum line and the filling regulated by means of a screw clamp to reduce foaming as much as possible. The beer and indicator are mixed by inverting the test tube or bottle and the long tube (B) inserted in a water seal through which CO_2 can escape. The temperature of the beer is immediately brought up to that of the room. If the beer is contained in clear, white bottles it is only necessary to chill the beer, remove the closure and pass a slow stream of CO_2 into the neck. The required amount of indicator is added and a one-hole rubber stopper with a bent glass tube leading into a water seal fitted.

After 3 or 24 hours the tubes in the water seal are clamped to exclude air. The triplicate tests with each indicator are compared to see whether they are the same in colour intensity. One of the three samples is then treated with a small quantity of hydrogen peroxide to bring the dye to 100% oxidation and the remaining two are compared with this and with the blank (beer that has not been treated with indicator), in a similar test container. The percentage decoloration of each indicator is thus approximated. Knowing the p_H of the sample under investigation the E_h may be calculated from Table 248. If the p_H value is 4·5 the E_h and r_H may be read directly from Table 249.

TABLE 248.—VARIATION OF E_0 AT DIFFERENT p_H VALUES (S. LAUFER)

p_H	Cresyl blue	Thionine	Methylene blue	Indigo tetra-sulphonate	Indigo tri-sulphonate	Indigo di-sulphonate	Hydrogen electrode
3·0	+0·312	+0·292	+0·267	+0·186	+0·150	+0·108	−0·134
3·2	0·295	0·275	0·248	0·171	0·137	0·096	0·143
3·4	0·278	0·277	0·232	0·159	0·125	0·084	0·152
3·6	0·262	0·238	0·215	0·147	0·113	0·072	0·161
3·8	0·243	0·222	0·198	0·135	0·101	0·060	0·170
4·0	0·228	0·205	0·180	0·123	0·096	0·048	0·179
4·2	0·212	0·191	0·163	0·112	0·087	0·036	0·188
4·4	0·194	0·177	0·146	0·100	0·065	0·024	0·197
4·6	0·177	0·163	0·129	0·088	0·053	0·012	0·206
4·8	0·160	0·150	0·112	0·076	0·041	0	0·215
5·0	0·147	0·137	0·098	0·064	0·029	−0·012	0·224
5·2	0·133	0·126	0·087	0·052	0·017	0·024	0·233
5·4	0·123	0·117	0·075	0·040	0·005	0·036	0·242
5·6	0·109	0·108	0·064	0·028	−0·007	0·048	0·251
5·8	0·098	0·100	0·054	0·014	0·019	0·060	0·260
6·0	0·088	0·093	0·046	0·002	0·031	0·072	0·269

The values for K in Table 249 were calculated from the equation $E_h = E_o + 0{\cdot}03 \log \frac{[Ox]}{[Re]}$, by substituting the estimated percentage decoloration of the indicator. Thus if it was judged that colour intensity was reduced by 2% K would become 0·051 volt.

$$E_h = E_o + 0{\cdot}03 \log \frac{98}{2} = E_o + 0{\cdot}051$$

The variations in E_0 at different p_H values are given in Table 248. To these the appropriate values of K must be added to obtain the E_h and r_H values corresponding with the percentage oxidation or reduction observed. The resulting figures at p_H 4·5 for three indicators are given in Table 249.

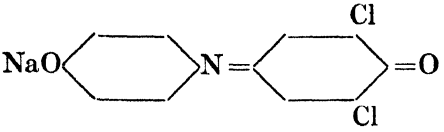
TABLE 249.—VARIATION OF POTENTIAL AND CORRESPONDING r_H AT 30° C. WITH PERCENTAGE OXIDATION AND REDUCTION AT p_H 4·5 (S. LAUFER)

Oxidation-reduction per cent.		Tint	K volts	Thionine		Methylene blue		Indigo tetra-sulphonate	
				E_h	r_H	E_h	r_H	E_h	r_H
1	99	almost full	+0·060	0·230	16·7	0·198	15·6	0·155	14·2
2	98		0·051	0·221	16·4	0·189	15·3	0·146	13·9
5	95		0·038	0·210	15·9	0·176	14·8	0·133	13·4
10	90		0·029	0·199	15·6	0·167	14·6	0·124	13·1
15	85		0·023	0·193	15·5	0·161	14·4	0·118	13·0
20	80		0·018	0·188	15·3	0·156	14·2	0·113	12·8
25	75	three-quarters	0·014	0·184	15·1	0·152	14·1	0·109	12·6
30	70		0·011	0·181	15·0	0·149	14·0	0·106	12·5
40	60		0·005	0·175	14·8	0·143	13·8	0·100	12·3
50	50	half-colour	0	0·170	14·7	0·138	13·6	0·095	12·2
60	40		−0·005	0·165	14·5	0·133	13·4	0·090	12·0
70	30		0·011	0·159	14·3	0·127	13·2	0·084	11·8
75	25	one quarter	0·014	0·156	14·2	0·124	13·1	0·081	11·7
80	20		0·018	0·152	14·1	0·120	13·0	0·077	11·6
85	15		0·023	0·147	13·9	0·115	12·8	0·072	11·4
90	10		0·029	0·141	13·7	0·109	12·6	0·066	11·2
95	5		0·038	0·132	13·5	0·100	12·4	0·057	11·0
98	2		0·051	0·119	13·0	0·087	11·9	0·044	10·5
99	1	almost colourless	0·060	0·110	12·7	0·078	11·6	0·035	10·2

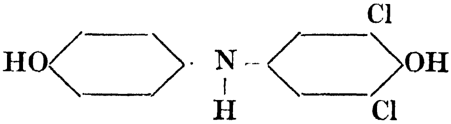
(705) Reducing Activity of Beer.

Hartong suggested a frankly empirical method for measuring the " reducing activity " of beer by means of an indicator which is decolored at a comparatively high oxidation-reduction potential. The indicator, 2 : 6-dichlorophenol indophenol, is practically insensitive to dissolved oxygen and is rapidly reduced by constituents of beer which are susceptible to oxidation by air. Decoloration is increasingly slow with beers which have been more fully aerated and are, on that account, less stable. The method is of considerable value as a quick routine process for determining the state of oxidation of beer and for forecasting its stability. It avoids or, at least, greatly reduces the main difficulties and uncertainties associated with potentiometric and colorimetric determinations of oxidation-reduction potential, the disturbing effects of free oxygen or the time required for decoloration of such an indicator as methylene blue. It cannot, however, claim the accuracy of potentiometric measurements with an electrode, such as that already described, which removes the objections in regard to contact with air and adsorption of oxygen. There is little doubt that, when a standard method is adopted, it will be electrometric and that the results will be expressed in terms of E_h and p_H.

The indicator is used in the sodium-quinonoid form, known also as 2 : 6-dichlorobenzenone indophenol.



This indicator is blue in solutions above p_H 6. The undissociated acid is red in solutions below p_H 4·6. Both these are oxidised forms. The reduced leuco-compound is phenolic, its acid form being



The indicator is characterised by the following constants :

E_0 at p_H	5, + 0·366.	r_H at 25° C.,	22·2
,,	7, + 0·217.	,,	21·3
,,	9, + 0·089.	,,	21·0

Hartong carries out the test in beer made alkaline with caustic soda, because decoloration is more clearly marked with blue than red. A 0·002 N. solution of the indicator is made by dissolving 58 mgm. in 100 ml. water. 5 ml. of this solution, with 1 ml. N.NaOH, are placed in a stoppered bottle of about 110 ml. capacity. This is then filled with the wort or beer to be tested, and the time required for decoloration, as compared with a control bottle containing beer only, is determined with a stop-watch. If this time is denoted by t seconds, the result is expressed as the "decoloration number" from the expression $\frac{30 \text{ minutes}}{t}$. If more than 30 minutes are required, the decoloration number is regarded as zero. Higher figures on this empirical scale indicate more rapid decoloration and denote greater reducing activity or lower state of oxidation.

The following are examples of Hartong's results. The first series includes three beers which have been subjected to increasing contact with air. The "D.N." values fall on a curve which is almost parallel with that for the corresponding oxidation-reduction potentials. They might consequently be used as a rapidly obtained, approximate measure of the latter. The second series, determined during the course of a brew, indicates the development of reducing activity during boiling with hops, its subsequent fall as a result of oxidation and increase during fermentation.

Hopped wort,	E = − 0·002 volt	"D.N."	90
Beer 1	E = + 0·138 ,,	,,	26
,, 2	E = + 0·158 ,,	,,	16
,, 3	E = + 0·208 ,,	,,	3–4
Mash tun wort		,,	7·8
Last wort		,,	15·6
Boiled wort		,,	72
Cooled wort (exposed to air 36 hours)		,,	20
Before fermentation		,,	12
After fermentation		,,	28·6

(706) The "Indicator-Time" Test.

A rapid routine method, similar in principle to that of Hartong, but more strictly standardised, was worked out at the Wallerstein Laboratories, New York. As described by Gray and Stone,[8] the test is carried out at the p_H of the sample, and not in alkaline solution. Decoloration is carried to 80%. A 0·005 molar solution of 2 : 6-dichlorobenzenone indophenol is prepared and standardised weekly. The standard strength is 145 mgm. per 100 ml. Rather more than 150 mgm. of powdered indicator is dissolved in hot water by stirring until cool. The solution is washed into a 100-ml. flask, made up to the mark, filtered through a Whatman 40 paper and 10 ml. pipetted into a 50-ml. Erlenmeyer flask for standardisation. 4 ml. potassium iodide solution (5 gr. in 20 ml.) and 2 ml. of 1 : 6 sulphuric acid are added to this. The mixed solution is then titrated with 0·01 N. thiosulphate from a 25-ml. burette, using 1 ml. of starch as indicator towards the end of titration. The strength of the indicator is calculated by multiplying the number of ml. of thiosulphate required by 14·5. This gives mgm. indicator per 200 ml. solution. Dilute with water to give a strength of 150 mgm. per 100 ml. to allow for slight oxidation.

The test is carried out by placing 10 ml. of the beer at 25° C. in three uniform test-tubes. The beer may be partially degassed to avoid foaming. Distilled water is placed in two similar tubes, and 80% decoloration standard in a sixth. This is prepared by adding, from an accurate capillary pipette, 0·05 ml. of standardised indicator to 10 ml. of phosphate buffer, p_H 4·4. To one of the tubes containing the sample add 0·25 ml. of 0·005 M indicator. Invert the tube to mix and start a stop-watch. This tube is placed in the front central hole of a six-hole comparator, with a tube of water behind it. On the left is a tube of beer, with water behind, and on the right is another tube of beer with 80% indicator behind. Note the time when the colour of the central tubes matches that of the tubes on the right. This time in seconds is the Indicator-Time Test Value.

Values obtained with beer vary from 50 seconds to 3,000 seconds or more. A close connection has been found with colloidal stability and flavour changes. The results can be correlated with the previous history of the beer in respect of contact with air, which raises the I.T.T. value. These values bear a logarithmic relation with r_H values determined colorimetrically. They both measure the same factors, but the I.T.T. values eliminate the effects of air, which immediately affect the reduced indicators. No attempt is made to convert I.T.T. values to the corresponding r_H, but a difference of 500 seconds was found to compare with an r_H difference of only 0·5 unit, after 5 hours' aeration. A linear relation was found between the percentage decoloration and the logarithm of the time. It is convenient to record the shorter time for 50% decoloration with highly oxidised samples and extrapolate for 80%. The comparison standard is then made by adding 0·125 ml. of indicator to 10 ml. of buffer solution. This can be used with beers having an I.T.T. value over 1,000. The resulting value multiplied by 7 is a close approximation to the I.T.T. value.

Permanent standards, which are stable for at least a year in sealed Pyrex test-tubes, can be made from

Solution A.	Roseo cobaltic chloride ($CoCl_3 \cdot 5NH_3 \cdot H_2O$)	5 grams
	Ammonium hydroxide (concentrated)	100 ml.
	Water to	1 litre
Solution B.	Copper sulphate ($CuSO_4 \cdot 5H_2O$) ..	5 grams
	Ammonium hydroxide (concentrated)	100 ml.
	Water to	1 litre

For the 80% standard mix 230 ml. of A and 70 ml. of B with 70 ml. NH_4OH.

For the 50% standard mix 540 ml. of A and 150 ml. of B with 31 ml. NH_4OH.

The following are examples of results :—

Beer from full tank, containing 1·6 ml. air per bottle	I.T.T.	167
Beer from later bottling, containing 2·7 ml. air per bottle	„	446
Wort before fermentation	„	150
Beer at end of fermentation	„	65
Storage 10 days	„	200
Storage 3 weeks	„	330
Bottled beer	„	420

NON-BIOLOGICAL TURBIDITIES

(707) Haze Formation in Beer.

All filtered beers become cloudy in time on account of the precipitation of colloidal constituents and quite irrespective of the presence of micro-organisms. The particles forming a haze are usually so minute that they pass through a filter paper more readily than the smallest micro-organisms and it is frequently possible to distinguish in this way between non-biological and biological turbidities. A small centrifuge is useful for obtaining a microscopical preparation, but some experience is required to distinguish between the more minute coccus forms of bacteria and spherical amorphous particles. The latter are frequently soluble in caustic soda solution or ether, and beer rendered cloudy by their presence can generally be clarified by addition of these reagents or by warming.

The quantity of suspended material required to cause cloudiness in beer is very small. Helm[9] quoted analyses of a large number of samples by Jessen-Hansen and Vimpel, in which 5·4 to 36 mgm. with an average of 13·4 mgm. per litre were found. Its composition is also very varied, the nitrogen content of these samples ranging from 5·9 to 9·9%, with an average of 8·6%, equivalent to 36·9 to 61·9% of protein, with an average of 53·8%. The ash content also varies greatly, one analysis showing 54·5% of protein and 1·5% of ash, of which 45% consisted of SiO_2 and 9% of Fe_2O_3, while other analyses have given 11% of ash and higher figures. Tannin is also present and, probably, carbohydrate, while the colour is brown from pigment adsorbed from the beer.

Turbidity of this nature is frequently referred to in a general way as *protein haze*, to distinguish it from that due to the growth of micro-organisms. Pasteurisation is no safeguard against its occurrence and, indeed, tends to hasten the production of haze in beer stored at ordinary temperatures. It is more liable to be noticed in pasteurised than unpasteurised beers, because its formation in the latter may be masked by the growth of yeast. The haze formed in pasteurised beers is consequently often referred to as *pasteurisation haze*. Many beers rapidly become cloudy when chilled, while the production of haze during storage at ordinary temperatures is hastened in beers that have remained in contact with clean metal surfaces for quite short periods during bottling. Hence the terms *chill haze* and *metal haze* are frequently used.

Owing to the uncertainty that still remains in regard to the composition of the haze-forming materials, the variations shown by analysis and consequent failure to identify chill haze and oxidation haze as definite substances, Helm suggested a classification of non-biological turbidities in which some were distinguished according to the factors that cause the precipitation. The classification includes hazes that occasionally result from imperfectly converted starch and are still less frequently formed by precipitation of calcium oxalate crystals. It also differentiates turbidities that are of such a nature as to suggest some fault in brewing from others that ultimately appear in faultlessly produced beers.

Turbidities " normally " occurring in beers.

(*a*) Chill haze.
(*b*) Oxidation haze.

Turbidities due to beer defects.

(*a*) Starch or dextrin turbidities.
(*b*) Metal haze.
(*c*) Oxalate haze.

(708) Chill Haze and Oxidation Haze.

Beer that has become cloudy by cooling in ice can be clarified by warming up again. That is to say, chill haze is *reversible.* Beer that has become cloudy during storage at ordinary temperatures, whether it has been pasteurised or not, cannot as a rule be clarified by gently warming. This haze or pasteurisation haze is permanent or *irreversible.* Helm[9] pointed out that oxidation was the most important factor in precipitation of the irreversible haze, giving rise to poor " chemical stability " in beer. He therefore suggested that this haze should be described as *oxidation haze* to distinguish it from chill haze. The chemical reaction of oxidation takes place slowly at ordinary temperatures, but much more rapidly at higher temperatures, so that pasteurised beers become particularly susceptible to oxidation haze. There is no specific haze due to pasteurisation, this process merely accelerating and accentuating the precipitation that occurs in unpasteurised beers. When bottled beers are stored, the oxygen in the neck of the bottles is gradually absorbed and, presumably, combined with beer constituents. Helm, for example, found that bottles containing 2·8 ml. of oxygen per litre at filling, contained only 1·1 ml. after 4 months.

The formation of both chill haze and oxidation haze is accelerated and increased by the presence of air in bottled beer, the effect being more marked with oxidation haze. This is shown by De Clerck's[10] results in Table 250. Varying quantities of oxygen were added to a series of pasteurised bottled beers and these were then stored at 86° F. for the periods shown. The relative quantities of chill haze were determined nephelometrically after immersion of the bottles for one hour in ice water. The oxidation haze that had developed at 86° F. was measured in the same way, but without chilling. Nephelometric figures of 10–20 indicate a slight haze formation. The beers were definitely cloudy at 30. It will be noted that chill haze appeared more rapidly and more strongly with increasing quantities of oxygen. It was, however, readily produced in the absence of air and this particular beer became completely cloudy by chilling after 7 days' storage at 86° F. Quantities of oxygen less than 1 ml. per litre appeared to have no influence on oxidation haze, but it is necessary to bottle in the absence of air to prevent its ultimate development. The sensitiveness of the beer to chill haze in the absence of oxygen was, on the other hand, sufficient to render air-free bottling of little use as a preventive measure.

TABLE 250.—EFFECT OF AIR ON HAZE PRODUCTION IN PASTEURISED BEER (J. DE CLERCK)

(BEERS STORED AT 86° F.)

ml. oxygen per litre	Chill haze, days' storage					Oxidation haze, days' storage				
	2	7	16	25	35	1	7	16	25	35
0	2·8	80	180	230	300	1·4	1·5	2·0	2·7	3·6
0·4	8·1	76	140	210	260	1·4	1·6	1·8	2·3	3·5
0·75	43	82	182	255	300	1·5	1·9	2·0	2·8	4·8
1·50	54	91	215	330	335	1·5	1·5	1·9	2·2	3·5
3·0	98	140	250	355	380	1·4	1·8	2·2	4·5	17
5·0	70	170	330	445	480	1·7	1·8	3·5	26	65
10·0	63	140	410	465	530	1·8	2·0	15	95	180
20·0	40	135	410	460	485	1·9	2·2	52	164	275

Oxidation haze generally appears much later than chill haze, that is to say beers that are still brilliant at ordinary temperatures become cloudy when chilled. It may in consequence be of no significance unless the beers are exported or kept for a considerable time. Foaming during bottle filling reduces the tendency to its formation by removing air with the CO_2.

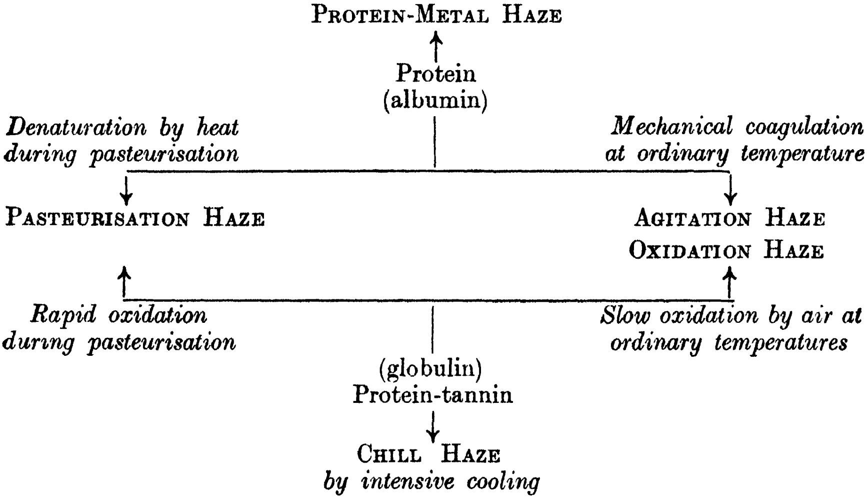
(709) Nature and Origin of Haze-forming Substances.

Haze production is due to many different causes and is influenced by both physical and chemical factors. Among those already mentioned are reduction in solubility of the haze-forming substance by chilling, increase in the tendency to haze under the influence of oxygen and precipitation of insoluble compounds by heavy metals. Among the factors that influence the denaturation and coagulation of proteins are high temperatures, agitation and changes in reaction (Vol. I, Sections 83–91). These all tend to promote haze formation, so that this may, possibly, be due in some cases to the influence of physical factors on colloidal constituents of the beer. Haze formation is accelerated by agitation during transport of bottled beer and is caused by reduction of acidity by traces of alkali in bottles or extraction of alkali from bottle glass or by increase of acidity through the development of bacteria. Storage at relatively high temperatures increases the haze formed on chilling. Intensive filtration reduces the sensitiveness to chilling.

It has not been found possible to determine the nature of the protein constituents of haze, but they are probably due to high molecular compounds. Chill haze is not due to coagulable nitrogen compounds, but its characteristics are so similar to those of the fine turbidity produced during wort cooling that there is little doubt that they are of the same nature and consist largely of protein-tannins. Hartong[11] suggested that the protein part was a proteose or "globulose," derived from malt globulin. Helm's experience was that the tendency to oxidation haze did not necessarily increase with the use of high nitrogen malt, but depended on the nature of the nitrogenous substances and would thus be influenced by the modification of the malt.

Lüers and Enders[12] support the protein-tannin origin of chill haze and oxidation haze, for which they obtained a considerable amount of indirect evidence. Thus, they found that the curves for turbidity production on cooling and for clarification on warming beers were identical with the corresponding curves given by such combinations as gelatin-tannin. Alternate production of haze by chilling and clarification by warming can be carried out repeatedly but, after a time, the beer will not become quite bright on warming. This they attribute to the oxidation of protein-tannin to protein-phlobaphene by the oxygen dissolved in the beer. The conclusion drawn from this and other experiments, showing slow oxidation at ordinary temperatures and rapid oxidation at higher, was that the irreversible haze produced during pasteurisation consisted essentially of protein-phlobaphene.

These authors emphasise the view that chill haze and oxidation haze are both protein-tannin turbidities in a classification in which they introduce a *true protein turbidity,* observed in German beers. This is occasionally deposited in the form of flocks at the bottom of bottles and differed from the protein-tannin hazes in that it consisted of protein only and was attributed to denaturation and coagulation by agitation of albumin that had escaped removal during boiling and fermentation. Helm remarked that no such deposit occurred in Danish beers and considered it unlikely that pure protein should be precipitated without adsorbing tannin or other colloidal constituents of the beer. Lüers' view of the origin and relationships of the various forms of haze is illustrated in the following scheme :—


PROTEIN-METAL HAZE
Protein
(albumin)
Denaturation by heat during pasteurisation
Mechanical coagulation at ordinary temperature
PASTEURISATION HAZE
AGITATION HAZE
OXIDATION HAZE
Rapid oxidation during pasteurisation
Slow oxidation by air at ordinary temperatures
(globulin)
Protein-tannin
CHILL HAZE
by intensive cooling

(710) Metal Hazes.

Beers usually contain minute traces of heavy metals, derived from the malt or from vessels and mains with which they have been in contact. The quantities may be so small as to render their determination by ordinary methods of analysis very difficult, but they can be detected by spectroscopy. The photospectrograph of the ash of beer, reproduced in Fig. 220 by the courtesy of Dr. S. Judd Lewis, shows the presence of copper, iron, lead and tin, together with the commoner elements sodium, potassium, magnesium, calcium, silicon, phosphorus and boron. The upper band in this figure is the spectrum given by the pure silver electrodes. The lower represents the combined spectrum of silver and ash and it is only the extra lines that express the analysis.

The presence of such minute quantities of metals as are normally present does not influence the brilliance of the beer, but an early production of haze is occasionally noticed with beers that have been allowed to remain for short periods in contact with copper or tin in the bottling plant or with the first few dozen bottles through carefully cleaned plant or mains of these metals. The figures in Table 251 show the quantities of copper found in a series of bottles taken throughout the run of a certain light pale ale and the times that elapsed before a haze was detected during storage at ordinary temperature.

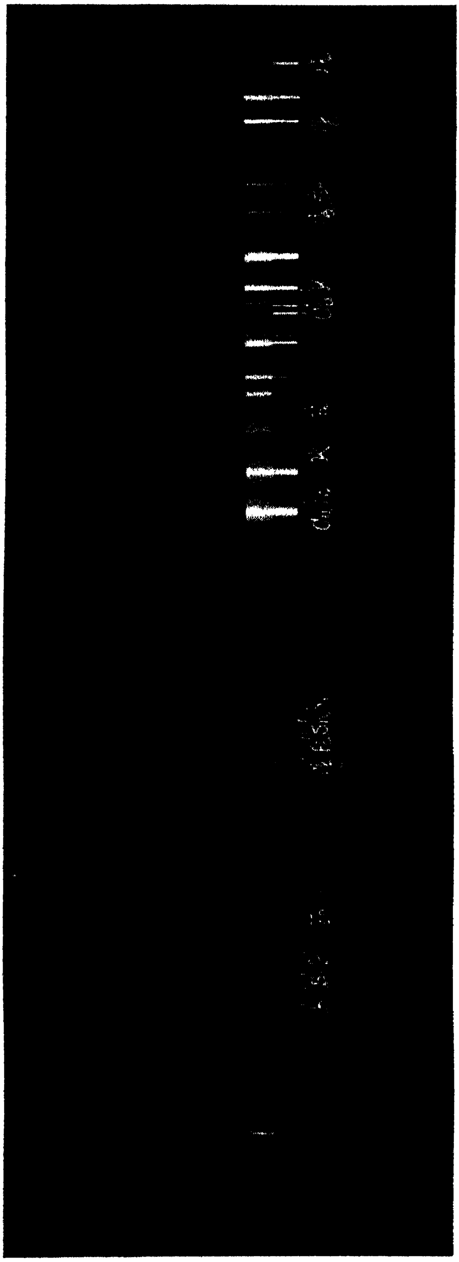


Fig. 220

SPECTRUM OF THE ASH OF NORMAL BEER (S. JUDD LEWIS)

The haze produced in the last two samples was associated with the growth of yeast and quantities of copper less than 0·75 mgm. per litre (0·05 grain per gallon) did not appear to reduce the colloidal stability of this particular beer, but greater amounts had a progressively accelerating effect on the production of haze in the beers stored at room temperature (about 62° F.).

TABLE 251.—EFFECT OF COPPER ON HAZE IN BOTTLES OF LIGHT PALE ALE TAKEN DURING COURSE OF BOTTLING

(STORED AT ABOUT 62° F.)

Copper in Beer	From tank	Samples analysed					
		1	2	3	4	5	6
Parts per million (mgm. per litre)	0·4	2·0	1·75	1·0	0·75	0·6	0·5
Grain per gallon ..	0·03	0·14	0·12	0·07	0·05	0·04	0·035
Haze detected, days ..	—	4	7	12	15	24	24

Tin, copper, iron and nickel are the metals with which beer is most likely to come in contact and all these can intensify permanent haze formation, the effects of equal quantities in solution being in the order given. They are all, with other heavy metals, capable of forming insoluble compounds with proteins and it is probable, though it has not been demonstrated with certainty, that the precipitate consists of such compounds. Their action may, however, be of a catalytic nature. The minimum quantity required to affect the colloidal stability of filtered bottled beers or the time in which the haze will become apparent varies with different beers and the state of oxidation of the metallic ion. According to Helm[13] quantities of 0·005 mgm. tin, 0·1 mgm. copper and 0·2 mgm. iron per litre (0·00035, 0·007 and 0·014 grain per gallon) could be provisionally regarded as unimportant with Danish lager beer. Quantities of copper of the order of 0·3 mgm. per litre may, however, be regarded as normal in most beers brewed in copper vessels and are usually without effect on brilliance. Idoux[14] found that 1 mgm. per litre ($\frac{1}{14}$ grain per gallon) of cuprous copper caused an immediate haze, while it took three days for haze to develop in the same beer containing 10 mgm. per litre of copper as a soluble cupric salt. The presence of such a reducing substance as a sulphite consequently accelerates the production of haze, particularly in pasteurised beer, with which 0·1 mgm. per litre of copper will cause a haze after some time. The reducing properties of beer cause early precipitation of copper and partly explain the very small quantity found in beers fermented in copper vessels. Solution is also hindered by the early formation of a scale or film on the copper. Hagues[15] obtained the results shown in Table 252 after addition of copper sulphate in varying quantities to an English bottled beer.

TABLE 252.—HAZE FORMATION IN BOTTLED BEER CAUSED BY ADDITION OF COPPER SULPHATE (G. HAGUES)

Grain per gallon of copper	Appearance after 10 days	Appearance after 1 month
0·30	Opalescent	Very opalescent
0·12	Slightly opalescent	,,
0·06	Brilliant	Opalescent
0·03	,,	,,
0·01	,,	Slightly opalescent
0·005	,,	,, ,,
Control	,,	Brilliant

Pure tin is very resistant to beer, but it rarely occurs in the pure state, the presence of impurities or contact with other metals facilitates attack. Traces may consequently be dissolved from tinned copper mains or vessels, particularly when these become worn, and even the faintest trace (above 0·005–0·01 mgm. per litre with different beers) may cause trouble. Copper surfaces are therefore safer than tinned copper, though the latter may be preferable when in perfect condition.

Matthews[16] stated that the iron content of beers might vary between 0·161 and 0·245 grain per gallon (2·3 and 3·5 mgm. per litre). A metallic flavour was noticeable if the higher quantity was exceeded. Helm found that 1 mgm. per litre produced a perceptible effect in Danish lagers. It has less effect on haze than copper. Helm also tested the effects of 1 mgm. per litre of aluminium, nickel, lead, zinc and chromium on haze and found that nickel only was to be feared at this concentration and its influence was much smaller than that of corresponding quantities of tin, copper or iron. Hagues found that 0·1 and 0·5 grain per gallon (1·4 and ·7 mgm. per litre) of nickel produced opalescence in bottled beers in 20 and 2 days, respectively. Traces of iron in sugar or caramels, added to beer in normal proportions, may cause haze.

No difficulty occurs when beer is running continuously through tin or copper mains and plant, but comparatively short periods of rest in contact with the metal are sufficient to permit of attack, especially if the metal has recently been cleaned with soda or acid. Haze production is usually accompanied by a slight metallic flavour in the beer, but the quantity of metal concerned can rarely be such as would be considered any danger to health. Dark beers are less liable than pale beers to show a metal haze, on account of the greater quantity of such protective colloids as melanoidins contained in them.

(711) Other Forms of Turbidity.

It occasionally happens that when two bright beers are blended the mixture becomes turbid. This may be due to several causes. The acidity of an old beer may upset the equilibrium of colloidal systems in a newer and cause protein precipitations, or excess of hop tannin in one may combine with proteins in the other. In some cases a haze is caused by separation of resin particles derived from the hops and, occasionally, from defective pitch linings of casks or storage vessels. A hop-resin haze gives a very astringent flavour. It can be dissolved in ether, as well as caustic soda.

Crystals of calcium oxalate are not infrequently seen when beer sediments are microscopically examined, the oxalic acid being derived from the malt. Calcium oxalate is almost insoluble in water, but dissolves to the extent of about 60 mgm. per litre in 1048 wort. The solubility is decreased to about 20 mgm. per litre in beer, so that the possibility of crystallisation occurs during fermentation and storage. It occasionally contributes to the formation of haze in bottom fermentation beers, through adsorption of oxalate ions on highly dispersed colloid particles. The adsorption complex develops into a calcium oxalate crystal, surrounded by coagulated protein, under the influence of agitation and low temperature (Geys[17]).

Turbidity due to the presence of starch or high molecular dextrins is liable to occur as a result of excessive temperature in the mash or sparge or through precipitation of dextrins as the alcohol content in beer increases. Haze of this type is not often encountered, but is very stubborn when it does occur. It may be identified by the colour given with iodine solution and is combated by use of highly diastatic malt extracts.

Occasionally a haze formed in beer by proteins is exceedingly stubborn, will not disappear by deposition during storage and resists the action of finings. In some cases this may be traced to the use of under-modified malts or unduly prolonged sparging with alkaline liquor. Turbidities of this kind are not the same as chill haze formed in bright beer, but they may be indistinguishable in composition. They are due to faults in materials or brewing and may be so difficult to remove that bright beer is hardly obtainable.

(712) Measurement of Haze.

Investigation of chill and oxidation hazes has necessarily involved elaboration of methods for measuring their intensity which are also of service for control purposes in the brewery or bottlery. Such terms as opalescent, cloudy, hazy, etc., are not sufficiently precise and advantage has been taken of the Tyndall effect produced by liquids containing colloid particles (Vol. I, Section 80). Several methods for measuring haze intensity by means of such elaborate instruments as photometers or nephelometers are available and a simple process, giving results comparable with the Pulfrich photometer, was described by Helm.[18] This consists in adding increasing quantities of barium sulphate to bottles containing clear beer of the same colour as that to be tested. A 0·025 molar suspension of $BaSO_4$ is prepared by mixing 10 ml. of 0·50 molar $BaCl_2$ and 10 ml. 0·55 molar K_2SO_4 and making the volume to 200 ml. One drop added to 350 ml. (the usual Continental-size beer bottle) of beer or water gives a turbidity number of 10 and so on. The test is carried out with white bottles or cylinders, 10 in. by 2 in., illuminated in such a way as to give a Tyndall effect, which causes a turbid beer to appear milky and a bright beer quite dark. The cylinders are placed in a colour comparator, consisting of an internally blackened box, with a horizontal aperture about 3 in. broad along the front. The blackened back of the box is about twice the height of the front or of the cylinders, which are illuminated from above by diffused daylight or by three cylindrical 60 W. lamps at the upper edge of the black background. Cylinders of beer, to which increasing quantities of the $BaSO_4$ suspension have been added, thus form a scale with which the beer to be tested is compared by observation from 1½ to 2 yards in front of the comparator. Readings obtained in this way are given in Table 253, in comparison with Pulfrich photometer readings. This method has an advantage over photometer measurements, in that it can be applied to bottled beers without opening them and thus introducing oxygen.

If the beer has turbidities, other than chill haze, they are measured and deducted from the value obtained at 32° F. The difference is an expression of the degree of chill turbidity. Should it be required to obtain a measure of the tendency of a beer to throw a haze on chilling, rather than of its quantity or intensity under standard conditions, this can be done by placing the bottles in ice and water at 32° F. or in brine at 23° F. and examining them at intervals of 5 minutes until a distinct haze can be detected in a strong light. The number of minutes required for this haze to appear may be taken as a measure of the chill-proof character of the beer. (Kretschmer and Emslander.[19])

TABLE 253.—TURBIDITIES COMPARED BY DIFFERENT METHODS (E. HELM)

$BaSO_4$ suspension 0 025 molar per 350 ml. beer	Turbidity number Helm	Turbidity percentage in the Pulfrich nephelometer	Roughly estimated
1 drop	10	5	Clear
2 drops	20	10	,,
5 ,,	50	23	Sl. opalescent
10 ,,	100	54	Opalescent
20 ,,	200	108	Turbid
40 ,,	400	215	,,

Petit[20] claimed that some idea of the time during which a beer will keep brilliant can be obtained by photometric measurements before and after pasteurisation. An increase on the Pulfrich photometer scale from about 10 in the original beer to 15 after pasteurisation would indicate a beer that is likely to retain its brilliance, while an increase of the order of from 10 to 40 would show that the beer is likely to have a short life.

Hartong[21] found pronounced differences in the degree of turbidity appearing in beer when saturated to 30% with ammonium sulphate and worked out a method based on the " ammonium sulphate precipitation limit " which should be useful in practice for predicting the chill sensitiveness of beer. Increasing quantities of saturated ammonium sulphate solution are added to a series of cylinders containing 10 ml. of the beer. The liquids are mixed and examined after 15 minutes. The ammonium sulphate precipitation limit is calculated from that cylinder in which an opalescence or Tyndall effect can first be detected, and given as the number of millilitres of saturated ammonium sulphate in 10 ml. of the mixed solution. If this is 0·85 ml. the Am_2SO_4 precipitation limit is $\frac{0{\cdot}85 \times 10}{10{\cdot}85} = 0{\cdot}78$. The greater this figure, the more stable will the beer be.

Since the onset of haze is hastened by the shaking to which beer is subjected in transit and particularly during export, methods have been developed to submit beers to continual shaking in mechanically operated cupboards. Helm[9] described two cupboards which are used in breweries for testing export beers. One is rocked like a reversed pendulum around an axis beneath the cupboard, making about 12 oscillations per minute. The second type, which was devised by Krause, is moved up and down vertically about 190 times a minute. The bottles are supported horizontally by rubber cylinders and turbidity measurements are made after shaking 10, 20, 40 and 60 days at a constant temperature of 68° F. The turbidity increases more rapidly in the hopping than in the rocking cupboard. In the absence of elaborate testing apparatus of this kind, the bottles may be placed on the shaking screens in the brewery mill room. Table 254 provides an example of the use of the rocking cupboard and shows the effect of pasteurising time on oxidation haze.

TABLE 254.—EFFECT OF PASTEURISATION ON OXIDATION HAZE (E. HELM)
(PILSENER BEER, 1043)

Pasteurisation	Oxidation haze after shaking in rocking cupboard for — days			
	10	20	40	60
20 minutes at 145° F.	19	53	90	200
120 minutes at 145° F.	31	88	123	250

(713) Summary.

The influence of air or oxygen on the stability of beer and its physical properties is very great, but its study has been hampered by lack of methods for the quantitative measurement of absorbed oxygen in wort and beer. In 1933 De Clerck showed that the biological and colloidal stability of beer, as well as its flavour, could be related to measurements of its oxidation-reduction potential, and valuable practical results have since been obtained by this method. These have, for example, emphasised the dangers of access of air to beer during bottling, and have led to improvements in plant and technique by which this could be minimised. Beer has a slight reducing power, and reactions between some of its constituents and dissolved oxygen result in intensification of the tendency to haze formation and deterioration of flavour. These reactions are accelerated by pasteurisation so that the tendency to haze formation is increased. The growth of yeast is increased in the presence of oxygen, so that beer bottled with a high air content throws a yeast deposit much more quickly than beer from which air has been excluded. As oxygen is utilised during the early stages of fermentation, the reducing capacity of the wort decreases until, at a certain point, yeast growth ceases. During storage, oxygen dissolved in the beer is similarly utilised by yeast, which again ultimately ceases to multiply. Yeast growth is associated with a high oxidation-reduction potential, and its inhibition with a low potential. It can be mathematically demonstrated that the oxidation-reduction potential of a known reversible oxidation-reduction system, in which the relation between E_h and p_H is comparable with that of the hydrogen electrode, may be measured in terms of a hypothetical pressure of hydrogen gas at the electrode. This pressure is expressed in atmospheres to represent the quantity of hydrogen, equivalent in reducing tendency to that of the system. This method of expression should not strictly be applied to beer, since the nature of the existing oxidation-reduction systems is unknown, but it is used in practice and has been found very convenient. The hydrogen pressure corresponding with the oxidation-reduction potential of beer is very low, and has to be expressed by such a cumbersome figure as $\frac{1}{10,000,000,000,000,000}$ atm. Figures of this order would be quite intolerable in use and, consequently, it is usual to adopt a convention analogous to that used for expressing hydrogen ion concentration in terms of p_H. The fraction is equivalent to $\frac{1}{10^{16}}$, which may be written 10^{-16}. This gives the basis of the r_H scale, according to which the pressure may be expressed as the logarithm of the fraction with the sign changed. The beer taken as an example has an r_H of 16. This is a moderately low r_H. A beer containing more air would have an r_H of 18 or 20, in which case it would be very susceptible to attack by yeasts. As these developed and absorbed the oxygen, the r_H of the beer would fall to 12 or 11, at which r_H yeast cannot grow.

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SPECIFIC GRAVITY AND EXTRACT TABLE.

The following Table is based on those compiled by Dr. Plato for the German Imperial Commission (Vol. I, Section 242) and refers to apparent specific gravities, as determined in the usual manner by weighing in a specific gravity bottle in air or by means of a saccharometer. Cane sugar % wt./vol. and % wt./wt. represent grams per 100 ml. and grams per 100 grams of solution respectively. The percentages by weight in column 6, corresponding with the specific gravities at 60° F. given in column I, were computed by interpolation from Plato's table for true specific gravities at 15°/15° C. and 16°/15° C., corrected to 60°/60° F. and then brought to 60°/60° in air by add. (sp. gr. —1) × 0·00121. The cane sugar weight percentages were converted to volume percentages and the solution divisors calculated. The column headed Plato gives the specific gravities in air at 20°/20° C. related to the cane sugar weight percentages and, with the latter, corresponds with the Plato Table commonly used in breweries and laboratories where 20° C. is the standard temperature. The column headed Balling similarly gives the specific gravities at 17·5°/17·5° C. from the Balling Table corresponding with the same sugar percentages. These specific gravities cannot accurately correspond with those at 60°/60° F. and 20°/20° C. on account of the errors in Balling's Table. The following densities were used in the calculations :—

Water at 15°/4° C.	... ...	0·999126
60° F./4° C.	... ...	0·999035
20°/4° C.	... ...	0·998234

SPECIFIC GRAVITY CONVERSION TABLE FOR CANE SUGAR SOLUTIONS.

	BRITISH UNITS.			PLATO		BALLING	BAUMÉ.
Sp. Gr. 60 °F.	Brewers' pounds	Cane Sugar % wt./vol.	Solution Divisor	Sp. Gr 20° C.	Cane Sugar % wt./wt. Degrees Brix.	Sp. Gr. 17·5° C.	Modulus 145
1002·5	0·9	0·643	3·888	1·00250	0·641	1·00256	0·36
1005·0	1·8	1·287	3·885	1·00499	1·281	1·00513	0·72
1007·5	2·7	1·932	3·882	1·00748	1·918	1·00767	1·08
1010·0	3·6	2·578	3·879	1·00998	2·552	1·01021	1·43
1012·5	4·5	3·225	3·876	1·01247	3·185	1·01274	1·78
1015·0	5·4	3·871	3·875	1·01496	3·814	1·01528	2·14
1017·5	6·3	4·517	3·874	1·01745	4·439	1·01776	2·48
1020·0	7·2	5·164	3·873	1·01993	5·063	1·02025	2·83
1022·5	8·1	5·810	3·872	1·02242	5·682	1·02273	3·17
1025·0	9·0	6·458	3·871	1·02490	6·300	1·02523	3·52
1027·5	9·9	7·107	3·869	1·02740	6·917	1·02776	3·86
1030·0	10·8	7·755	3·868	1·02989	7·529	1·03027	4·20
1032·5	11·7	8·405	3·867	1·03238	8·140	1·03277	4·54
1035·0	12·6	9·054	3·866	1·03486	8·748	1·03527	4·88
1037·5	13·5	9·703	3·865	1·03736	9·352	1·03775	5·22
1040·0	14·4	10·354	3·863	1·03985	9·956	1·04024	5·55
1042·5	15·3	11·003	3·862	1·04234	10·554	1·04273	5·88
1045·0	16·2	11·652	3·862	1·04481	11·150	1·04523	6·21

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	BRITISH UNITS.			PLATO.		BALLING.	BAUMÉ.
Sp. Gr. 60° F.	Brewers' pounds	Cane Sugar % wt./vol.	Solution Divisor	Sp. Gr. 20° C.	Cane Sugar % wt./wt. Degrees Brix.	Sp. Gr. 17·5° C.	Modulus 145
1047·5	17·1	12·303	3·861	1·04731	11·745	1·04773	6·54
1050·0	18·0	12·953	3·860	1·04979	12·336	1·05022	6·87
1052·5	18·9	13·604	3·859	1·05227	12·925	1·05269	7·20
1055·0	19·8	14·255	3·858	1·05476	13·512	1·05515	7·52
1057·5	20·7	14·907	3·857	1·05726	14·097	1·05760	7·84
1060·0	21·6	15·560	3·856	1·05975	14·679	1·06005	8·16
1062·5	22·5	16·213	3·855	1·06224	15·259	1·06252	8·48
1065·0	23·4	16·866	3·854	1·06472	15·837	1·06500	8·80
1067·5	24·3	17·519	3·853	1·06720	16·411	1·06747	9·12
1070·0	25·2	18·173	3·852	1·06970	16·984	1·06995	9·44
1072·5	26·1	18·827	3·851	1·07218	17·554	1·07244	9·75
1075·0	27·0	19·482	3·850	1·07467	18·122	1·07494	10·06
1077·5	27·9	20·135	3·849	1·07717	18·687	1·07743	10·37
1080·0	28·8	20·791	3·848	1·07965	19·251	1·07990	10·69
1082·5	29·7	21·446	3·847	1·08213	19·812	1·08237	11·00
1085·0	30·6	22·101	3·846	1·08462	20·370	1·08486	11·30
1087·5	31·5	22·758	3·845	1·08712	20·927	1·08737	11·61
1090·0	32·4	23·414	3·844	1·08960	21·481	1·08986	11·91
1092·5	33·3	24·071	3·843	1·09209	22·033	1·09235	12·21
1095·0	34·2	24·726	3·842	1·09457	22·581	1·09481	12·51
1097·5	35·1	25·384	3·841	1·09707	23·129	1·09730	12·81
1100·0	36·0	26·041	3·840	1·09956	23·674	1·09980	13·11
1102·5	36·9	26·700	3·839	1·10204	24·218	1·10230	13·41
1105·0	37·8	27·360	3·838	1·10454	24·760	1·10480	13·71
1107·5	38·7	28·019	3·837	1·10703	25·299	1·10730	14·00
1110·0	39·6	28·679	3·836	1·10952	25·837	1·10983	14·30
1112·5	40·5	29·339	3·834	1·11200	26·372	1·11235	14·59
1115·0	41·4	30·000	3·833	1·11450	26·906	1·11486	14·88
1117·5	42·3	30·660	3·832	1·11698	27·436	1·11735	15·17
1120·0	43·2	31·321	3·831	1·11947	27·965	1·11984	15·46
1122·5	44·1	31·981	3·830	1·12195	28·491	1·12231	15·74
1125·0	45·0	32·643	3·829	1·12445	29·016	1·12478	16·03
1127·5	45·9	33·305	3·828	1·12694	29·539	1·12729	16·31
1130·0	46·8	33·970	3·827	1·12944	30·062	1·12980	16·60
1132·5	47·7	34·632	3·826	1·13191	30·580	1·13228	16·88
1135·0	48·6	35·295	3·825	1·13441	31·097	1·13477	17·16
1137·5	49·5	35·958	3·824	1·13689	31·611	1·13723	17·44
1140·0	50·4	36·621	3·823	1·13938	32·124	1·13971	17·72
1142·5	51·3	37·285	3·822	1·14186	32·635	1·14221	18·00
1145·0	52·2	[illegible]	3·821	1·14435	33·145	1·14473	18·27
1147·5	53·1	[illegible]	3·820	1·14685	33·653	1·14727	18·55
1150·0	54·0	[illegible]	3·818	1·14934	34·160	1·14980	18·82